



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: C12N 15/57, C07K 14/47, C12N 9/64, C07K , C12Q 1/68, G01N 33/50	A2	(11) International Publication Number: WO 99/34004 (43) International Publication Date: 8 July 1999 (08.07.99)
(21) International Application Number: PCT/US98/27608 (22) International Filing Date: 24 December 1998 (24.12.98) (30) Priority Data: 60/070,112 31 December 1997 (31.12.97) US (71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street - R440, Emeryville, CA 94608 (US). (72) Inventors: XIN, Hong; Chiron Corporation, Intellectual Prop- erty - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US). GIESE, Klaus; Chiron Corporation, Intellectual Prop- erty - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US). (74) Agents: POTTER, Jane, E., R. et al.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).		(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: METASTATIC BREAST AND COLON CANCER REGULATED GENES (57) Abstract Gene sequences as shown in SEQ ID NO:1-18 have been discovered and isolated, and found to be significantly associated with metastatic spread of breast and colon cancer cells to other organs. Methods are provided for determining the risk of metastasis of a breast or colon tumor, which involve determining whether a tissue sample from a tumor expresses a polypeptide encoded by a gene as shown in SEQ ID NOS:1-18, or a substantial portion thereof. One of the gene sequences encodes a novel aspartyl protease termed CSP56, which can be used to provide reagents and methods for determining which tumors are likely to metastasize and for suppressing metastases of these tumors. Clinicians can use this information to predict which tumors will metastasize to other organs and to provide relevant therapies to appropriate patients.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/27608

Parent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 0848062	A	17-06-1998	JP 11069981 A	16-03-1999

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:17.

18. Claims: 1-34,36-40,42,43 (1,3,5,8,10,11,13,16,17,19,21,23, 25,27,29-32,34,36,39,40,42,43: only partly)

This eightieth group of inventions consists of the inventions as defined in claims 2, 4, 6, 7, 9, 12, 14, 15, 18, 20, 22, 24, 26, 28, 33, 37 and 38, which are each related to either of the sequences referred to as SEQ ID NO:18 and SEQ ID NO:19 (which is the amino acid sequence encoded by SEQ ID NO:18), as well as the inventions as defined in claims 1, 3, 5, 8, 10, 11, 13, 16, 17, 19, 21, 23, 25, 27, 29-32, 34, 36, 39, 40, 42 and 43, insofar the subject-matter of each of these latter claims concerns either of the sequences referred to as SEQ ID NO:18 and SEQ ID NO:19.

METASTATIC BREAST AND COLON CANCER REGULATED GENES

TECHNICAL FIELD OF THE INVENTION

5 This invention relates to methods for predicting the behavior of tumors and in particular, but not exclusively, to methods in which a tumor sample is examined for expression of a specified gene sequence which indicates propensity for metastatic spread.

BACKGROUND OF THE INVENTION

10 Despite use of a number of histochemical, genetic, and immunological markers, clinicians still have a difficult time predicting which tumors will metastasize to other organs. Some patients are in need of adjuvant therapy to prevent recurrence and metastasis and others are not. Distinguishing between these subpopulations of patients is not straightforward. Thus the course of treatment is not easily charted. There is therefore
15 a need in the art for new markers for distinguishing between tumors of differing metastatic potential.

SUMMARY OF THE INVENTION

20 It is an object of the invention to provide reagents and methods for determining which tumors are likely to metastasize and for suppressing metastases of these tumors. These and other objects of the invention are provided by one or more of the embodiments described below.

25 One embodiment of the invention is an isolated and purified protein having an amino acid sequence which is at least 85% identical to an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18. Percent identity is determined using a Smith-Waterman
homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.

30 Another embodiment of the invention is an isolated and purified polypeptide which consists of at least 8 contiguous amino acids of a protein having an amino acid

sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

Yet another embodiment of the invention is a fusion protein which comprises a first protein segment and a second protein segment fused to each other by means of a peptide bond. The first protein segment consists of at least 8 contiguous amino acids selected from an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

Still another embodiment of the invention is a preparation of antibodies which specifically bind to a protein with an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

Even another embodiment of the invention is a cDNA molecule which encodes an isolated and purified protein having an amino acid sequence which is at least 85% identical to an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1-18. Percent identity is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.

Another embodiment of the invention is a cDNA molecule which encodes at least 8 contiguous amino acids of a protein encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

Even another embodiment of the invention is a cDNA molecule comprising at least 12 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

Still another embodiment of the invention is a cDNA molecule which is at least 85% identical to a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18. Percent identity is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.

A further embodiment of the invention is an isolated and purified subgenomic polynucleotide comprising a nucleotide segment which hybridizes to a nucleotide

sequence selected from the group consisting of SEQ ID NOS:1-18 after washing with 0.2 X SSC at 65 °C.

Another embodiment of the invention is a construct comprising a promoter and a polynucleotide segment encoding at least 8 contiguous amino acids of a protein
5 encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18. The polynucleotide segment is located downstream from the promoter, wherein transcription of the polynucleotide segment initiates at the promoter.

Yet another embodiment of the invention is a host cell comprising a construct
10 which comprises a promoter and a polynucleotide segment encoding at least 8 contiguous amino acids of a protein encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

Even another embodiment of the invention is a recombinant host cell comprising a new transcription initiation unit. The new transcription initiation unit comprises in 5' to
15 3' order (a) an exogenous regulatory sequence, (b) an exogenous exon, and (c) a splice donor site. The new transcription initiation unit is located upstream of a coding sequence of a gene. The coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18. The exogenous regulatory sequence controls transcription of the coding sequence of the gene.

20 Still another embodiment of the invention is a polynucleotide probe comprising (a) at least 12 contiguous nucleotides selected from the group consisting of SEQ ID NOS:1-18 and (b) a detectable label.

Even another embodiment of the invention is a method for identifying a metastatic tissue or metastatic potential of a tissue. An expression product of a gene
25 comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-4, 6-13, and 15-18 is measured in a tissue sample. A tissue sample which expresses a product of a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 4, 11, 16, 17, and 18 or which does not express a product of a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:2,
30 3, 6, 7, 8, 9, 10, 12, 13, and 15 is identified as metastatic or as having metastatic

potential.

Still another embodiment of the invention is a method of screening test compounds for the ability to suppress the metastatic potential of a tumor. A biological sample is contacted with a test compound. Synthesis of a protein having an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-4, 6-13, and 15-18 is measured in the biological sample. A test compound which decreases synthesis of a protein encoded by a polynucleotide comprising SEQ ID NOS:1, 4, 11, 16, 17, or 18 or which increases synthesis of a protein encoded by a polynucleotide comprising SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, or 15 is identified as a potential agent for suppressing the metastatic potential of a tumor.

Another embodiment of the invention is a method of predicting propensity for high-grade or low-grade metastatic spread of a colon tumor. An expression product of a gene having a sequence selected from the group consisting of SEQ ID NO:16 and 17 is measured in a colon tumor sample. A colon tumor sample which expresses the product of SEQ ID NO:16 is categorized as having a high propensity to metastasize and a colon tumor sample which expresses the product of SEQ ID NO:17 is categorized as having a low propensity to metastasize.

Still another embodiment of the invention is a set of primers for amplifying at least a portion of a gene having a coding sequence selected from the group consisting of the nucleotide sequences shown in SEQ ID NOS:1-18.

Even another embodiment of the invention is a polynucleotide array comprising at least one single-stranded polynucleotide which comprises at least 12 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

A further embodiment of the invention is a method of identifying a metastatic tissue or metastatic potential of a tissue. A tissue sample comprising single-stranded polynucleotide molecules is contacted with a polynucleotide array comprising at least one single-stranded polynucleotide probe. The at least one single-stranded polynucleotide probe comprises at least 12 contiguous nucleotides of a nucleotide

sequence selected from the group consisting of SEQ ID NOS:1-4, 6-13, and 15-18. The tissue sample is suspected of being metastatic or of having metastatic potential. Double-stranded polynucleotides bound to the polynucleotide array are detected. Detection of a double-stranded polynucleotide comprising contiguous nucleotides selected from the group consisting of SEQ ID NOS:1-4, 11, 16, 17, and 18 or lack of detection of a double-stranded polynucleotide comprising contiguous nucleotides selected from the group consisting of SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, and 15 identifies the tissue sample as metastatic or of having metastatic potential.

The invention thus provides the art with a number of genes and proteins, which can be used as markers of metastasis. These are useful for more rationally prescribing the course of therapy for cancer patients, especially those with breast or colon cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Arbitrary primer-based differential display and confirmation by RNA blot analysis of different human breast cancer cell line. Figure 1A. Autoradiograph of a differential display gel depicting two bands of approximately 1.2 kb in size in the human breast cancer cell line MDA-MB-435. Differential display reactions were prepared and run in duplicates. Figure 1B. Northern blot analysis verifying the expression pattern in MDA-MB-435. cDNA isolated from the differential display gel hybridized to two transcripts of approximately 2.0 kb and 2.5 kb in size. Equal amounts of RNA in each lane were loaded as judged by staining of the membrane with methylene blue and hybridization of the membrane with a human β -actin probe.

Figure 2. Nucleotide sequence and deduced amino acid sequence of CSP56. Figure 2A. The 518 amino acid long sequence is shown in single-letter code below the nucleotide sequence of 1855 base pairs. The active site residue (D) and flanking amino acid residues characteristic of aspartyl proteases are underlined. The putative propeptide is boxed. The putative signal peptide at the N-terminus and the transmembrane domain at the C-terminus are underlined. Figure 2B. Expressed sequence tags extending the nucleotide sequence of CSP56 to 2606 base pairs in length. Figure 2C. Schematic representation of CSP56. SS, signal sequence; Pro, propeptide; TM transmembrane

domain. The asterisks indicate the active sites.

Figure 3. Multiple amino acid sequence alignment of CSP56 with other members of the pepsin family of aspartyl proteases. Identical amino acid residues are indicated by black boxes. The aspartyl protease active residues (D-S/T-G) are indicated by a bar on top. The cysteine residues characteristic for aspartyl protease in members of the pepsin family are indicated by asterisks. The putative membrane attachment domain is underlined. Gaps are indicated by dots. Cat-E, cathepsin E; Pep-A, pepsinogen E; Pep-C, pepsinogen C; Cat-D, cathepsin D.

Figure 4. CSP56 expression in primary tumor and metastases isolated from scid mice. Northern blot analysis using RNA isolated from primary tumors (PT) and metastatic tissues (Met) of mice injected with different human breast cancer cell lines. Equal amounts of RNA in each lane were loaded as judged by staining of the membrane with methylene blue and hybridization of the membrane with a human β -action probe.

Figure 5. CSP56 is up-regulated in patient breast tumor samples. Figure 5A. Northern blot analysis using RNA isolated from tumor and normal breast tissue from the same patient. Figure 5B. Northern blot analysis using RNA isolated from three different human breast tumor patients and normal breast tissue.

Figure 6. *In situ* hybridization analysis of CSP56 expression in breast and colon tumors. Adjacent or near-adjacent sections through normal breast tissue (A-C) and the primary breast tissue (D-F) of one patient and through normal colon tissue (G, H), the primary colon tumor (J, K), and the liver metastasis (L, M) of another patient. Sections A, D, G, J, and L were stained with haematoxylin and eosin (H & E). Sections B, E, H, K, and M were hybridized with the antisense CSP56 probe, and sections C and F were hybridized with the CSP56 sense control probe. d, lactiferous duct; f, fatty connective tissue; ly, lymphocytes; m, colon mucosa; met, metastatic tissue; PT, primary tumor; st, stroma; tc, tumor cells.

Figure 7. Expression of CSP56 in human tissues. RNA blot analysis depicting two CSP56 transcripts of 2.0 kb and 2.5 kb in various human tissues. sk. muscle, skeletal muscle; sm. intestine, small intestine; p.b. lymphocytes, peripheral blood lymphocytes.

DETAILED DESCRIPTION OF THE INVENTION

It is a discovery of the present invention that a number of genes are differentially expressed between cancer cells and non-metastatic cancer cells (Table 1). This
5 information can be utilized to make diagnostic reagents specific for the expression products of the differentially displayed genes. It can also be used in diagnostic and prognostic methods which will help clinicians in planning appropriate treatment regimes for cancers, especially of the breast or colon.

Some of the metastatic markers disclosed herein, such as clone 122, are up-
10 regulated in metastatic cells relative to non-metastatic cells. Some of the metastatic markers, such as clones 337 and 280, are down-regulated in metastatic cells relative to non-metastatic cells. Identification of these relationships and markers permits the formulation of reagents and methods as further described below. In addition, homologies to known proteins have been identified which suggest functions for the disclosed
15 proteins. For example, transcript 280 is homologous to human N-acetylglucosamine-6-sulfatase precursor, transcript 245 is homologous to bifunctional ATP sulfurylase-adenosine 5'-phosphosulfate kinase, and transcript 122 is homologous to human pepsinogen c, an aspartyl protease.

It is another discovery of the present invention that a novel aspartyl-type protease,
20 CSP56, is over-expressed in highly metastatic cancer, particularly in breast and colon cancer, and is associated with the progression of primary tumors to a metastatic state. This information can be utilized to make diagnostic reagents specific for expression products of the *CSP56* gene. It can also be used in diagnostic and prognostic methods which will help clinicians to plan appropriate treatment regimes for cancers, especially of
25 the breast and colon.

The amino acid sequence of CSP56 protein is shown in SEQ ID NO:19. Amino acid sequences encoded by novel polynucleotides of the invention can be predicted by running a translation program for each of the three reading frames for a particular polynucleotide sequence. A metastatic marker protein encoded by a polynucleotide
30 comprising a nucleotide sequence as shown in SEQ ID NOS:1-17, the CSP56 protein

shown in SEQ ID NO:19, or naturally or non-naturally occurring biologically active protein variants of metastatic marker proteins, including CSP56, can be used in diagnostic and therapeutic methods of the invention. Biologically active metastatic marker protein variants, including CSP56 variants, retain the same biological activities as the proteins encoded by polynucleotides comprising SEQ ID NOS:1-18. Biological activities of metastatic marker proteins include differential expression between tumors and normal tissue, particularly between tumors with high metastatic potential and normal tissue. Biological activity of CSP56 also includes the ability to permit metastases and aspartyl-type protease activity.

Biological activity of a metastatic marker protein variant, including a CSP56 variant, can be readily determined by one of skill in the art. Differential expression of the variant, for example, can be measured in cell lines which vary in metastatic potential, such as the breast cancer cell lines MDA-MB-231 (Brinkley *et al.*, *Cancer Res.* 40, 3118-29, 1980), MDA-MB-435 (Brinkley *et al.*, 1980), MCF-7, BT-20, ZR-75-1, MDA-MB-157, MDA-MB-361, MDA-MB-453, Alab and MDA-MB-468, or colon cancer cell lines Km12C and Km12L4A. The MDA-MB-231 cell line was deposited at the ATCC on May 15, 1998 (ATCC CRL-12532). The Km12C cell line was deposited at the ATCC on May 15, 1998 (ATCC CRL-12533). The Km12L4A cell line was deposited at the ATCC on March 19, 1998 (ATCC CRL-12496). The MDA-MB-435 cell line was deposited at the ATCC on October 9, 1998 (ATCC CRL 12583). The MCF-7 cell line was deposited at the ATCC on October 9, 1998 (ATCC CRL-12584).

Expression in a non-cancerous cell line, such as the breast cell line Hs58Bst, can be compared with expression in cancerous cell lines. Alternatively, a breast cancer cell line with high metastatic potential, such as MDA-MB-231 or MDA-MB-435, can be contacted with a polynucleotide encoding a variant and assayed for lowered metastatic potential, for example by monitoring cell division or protein or DNA synthesis, as is known in the art. Aspartyl protease activity of a potential CSP56 variant can also be measured, for example, as taught in Wright *et al.*, *J. Prot. Chem.* 16, 171-81 (1997).

Naturally occurring biologically active metastatic marker protein variants, including variants of CSP56, are found in humans or other species and comprise amino

acid sequences which are substantially identical to the amino acid sequences encoded by polynucleotides comprising nucleotide sequences of SEQ ID NOS:1-18. Non-naturally occurring biologically active metastatic marker protein variants can be constructed in the laboratory, using standard recombinant DNA techniques.

5 Preferably, naturally or non-naturally occurring biologically active metastatic marker protein variants have amino acid sequences which are at least 65%, 75%, 85%, 90%, or 95% identical to the amino acid sequences encoded by polynucleotides comprising nucleotide sequences of SEQ ID NOS:1-18 and have similar differential expression patterns, though these properties may differ in degree. Naturally or non-
10 naturally occurring biologically active CSP56 variants also have aspartyl-type protease activity. More preferably, the variants are at least 98% or 99% identical. Percent sequence identity is determined using computer programs which employ the Smith-Waterman algorithm using an affine gap search with the following parameters: a gap open penalty of 12 and a gap extension penalty of 1. The Smith-Waterman homology
15 search algorithm is taught in Smith and Waterman, *Adv. Appl. Math.* (1981) 2:482-489.

Guidance in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, such as DNASTAR software. Preferably, amino acid changes in biologically active metastatic marker protein variants are
20 conservative amino acid changes, *i.e.*, substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline,
25 phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cystine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. It is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid
30 with a structurally related amino acid will not have a major effect on the biological

properties of the resulting metastatic marker protein variant. For example, isolated conservative amino acid substitutions are not expected to have a major effect on the aspartyl protease activity of CSP56, especially if the replacement is not at the catalytic domains of the protease.

5 Metastatic marker protein variants also include allelic variants, species variants, muteins, glycosylated forms, aggregative conjugates with other molecules, and covalent conjugates with unrelated chemical moieties which retain biological activity. Covalent metastatic marker variants can be prepared by linkage of functionalities to groups which are found in the amino acid chain or at the N- or C-terminal residue, as is known in the
10 art. Truncations or deletions of regions which do not affect the expression patterns of metastatic marker proteins or, for example, the aspartyl protease activity of CSP56, are also biologically active variants.

A subset of mutants, called muteins, is a group of proteins in which neutral amino acids, such as serine, are substituted for cysteine residues which do not participate in
15 disulfide bonds. These mutants may be stable over a broader temperature range than naturally occurring proteins. See Mark *et al.*, U.S. Pat. No. 4,959,314.

Metastatic marker polypeptides contain fewer amino acids than full-length metastatic marker proteins. Metastatic marker protein polypeptides can contain at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, or
20 700 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:1; at least 8, 10, 12, 15, 25, 50, 75, 100, or 125 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NOS:2 or 9; at least 8, 10, 12, 15, 25, 50, 75, or 100 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NOS:3, 4, 5, 8, or 10; at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550,
25 600, 650, 700, 750, or 800 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:6; at least 8, 10, 12, 14, 25, 50, 55, or 60 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:7; 8, 10, 12, 15, 25, 50, 75, 100, 150, or 160 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:11; at least 8, 10, 12, 15, 25, 50, 75, 100, 125, or 130 contiguous amino acids
30 encoded by a polynucleotide comprising SEQ ID NO:12; at least 8, 10, 12, 15, 25, 50,

75, or 100 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:13; at least 8, 10, 12, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, or 300 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:14; at least 8, 10, 12, 15, 25, 50, 75, 100, or 150 contiguous amino acids encoded by a
5 polynucleotide comprising SEQ ID NO:15; at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, or 1100 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:16; or at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, or 500 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:17 in
10 the same order as found in the full-length protein or biologically active variant. CSP56 polypeptides can contain at least 8, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 23, 25, 28, 29, 30, 31, 32, 33, 35, 40, 50, 60, 75, 100, 111, 112, 120, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 or more amino acids of a CSP56 protein or biologically active variant. Preferred CSP56 polypeptides comprise at least amino acids
15 106-115, 105-116, 104-117, 100-120, 297-306, 296-307, 295-308, 290-320, 8-20, 7-21, 6-22, 1-30, 461-489, 460-490, 459-491, and 407-518 of SEQ ID NO:19. Polypeptide molecules having substantially the same amino acid sequence as the amino acid sequences encoded by polynucleotides comprising nucleotide sequences of SEQ ID NOS:1-18 thereof but possessing minor amino acid substitutions which do not
20 substantially affect the biological properties of a particular metastatic marker polypeptide variant are within the definition of metastatic marker polypeptides.

Metastatic marker proteins or polypeptides can be isolated from, for example, human cells, using biochemical techniques well known to the skilled artisan. A preparation of isolated and purified metastatic marker protein is at least 80% pure; preferably, the preparations are at least 90%, 95%, 98%, or 99% pure. Metastatic marker
25 proteins and polypeptides can also be produced by recombinant DNA methods or by synthetic chemical methods. For production of recombinant metastatic marker proteins or polypeptides, coding sequences selected from SEQ ID NOS:1-18 can be expressed in known prokaryotic or eukaryotic expression systems. Bacterial, yeast, insect, or
30 mammalian expression systems can be used, as is known in the art. Alternatively,

synthetic chemical methods, such as solid phase peptide synthesis, can be used to synthesize metastatic marker protein or polypeptides. Biologically active protein or polypeptide variants can be similarly produced.

5 Fusion proteins comprising contiguous amino acids of metastatic marker proteins of the invention can also be constructed. Fusion proteins are useful for generating antibodies against metastatic marker protein amino acid sequences and for use in various assay systems. For example, CSP56 fusion proteins can be used to identify proteins which interact with CSP56 protein and influence, for example, its aspartyl protease activity, its differential expression, or its ability to permit metastases. Physical methods, 10 such as protein affinity chromatography, or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can also be used for this purpose. Such methods are well known in the art and can also be used as drug screens.

A fusion protein comprises two protein segments fused together by means of a peptide bond. The first protein segment consists of at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, or 700 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:1; at least 8, 10, 12, 15, 25, 50, 75, 100, or 125 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NOS:2 or 9; at least 8, 10, 12, 15, 25, 50, 75, or 100 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NOS:3, 4, 5, 8, or 10; at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, or 800 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:6; at least 8, 10, 12, 14, 25, 50, 55, or 60 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:7; 8, 10, 12, 15, 25, 50, 75, 100, 150, or 160 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:11; at least 8, 10, 12, 15, 25, 50, 75, 100, 125, or 130 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:12; at least 8, 10, 12, 15, 25, 50, 75, or 100 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:13; at least 8, 10, 12, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, or 300 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:14; at least 8, 10, 12, 15, 25, 50, 75, 100, or 150 30

contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:15; at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, or 1100 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:16; or at least 8, 10, 12, 15, 25, 50, 75, 100, 150, 200, 250, 300, 350, 400, 450, or 500 contiguous amino acids encoded by a polynucleotide comprising SEQ ID NO:17, or at least 8, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 23, 25, 28, 29, 30, 31, 32, 33, 35, 40, 50, 60, 75, 100, 111, 112, 120, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, or 500 contiguous amino acids of a CSP56 protein. The amino acids can be selected from the amino acid sequences encoded by polynucleotides comprising SEQ ID NOS:1-18 or from a biologically active variants of those sequences. The first protein segment can also be a full-length metastatic marker protein. The first protein segment can be N-terminal or C-terminal, as is convenient.

The second protein segment can be a full-length protein or a protein fragment or polypeptide. Proteins commonly used in fusion protein construction include β -galactosidase, β -glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions.

These fusions can be made, for example, by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises coding sequences selected from SEQ ID NOS:1-18 in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available from companies that supply research labs with tools for experiments,

including, for example, Promega Corporation (Madison, WI), Stratagene (La Jolla, CA), Clontech (Mountain View, CA), Santa Cruz Biotechnology (Santa Cruz, CA), MBL International Corporation (MIC; Watertown, MA), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

5 Isolated metastatic marker proteins, polypeptides, biologically active variants, or fusion proteins can be used as immunogens, to obtain a preparation of antibodies which specifically bind to epitopes of metastatic marker protein. The antibodies can be used, *inter alia*, to detect metastatic marker proteins, such as CSP56, in human tissue, particularly in human tumors, or in fractions thereof. The antibodies can also be used to
10 detect the presence of mutations in metastatic marker protein genes, such as the *CSP56* gene, which result in under- or over-expression of a metastatic marker protein or in expression of a metastatic marker protein with altered size or electrophoretic mobility. By binding to CSP56, for example, antibodies can also prevent CSP56 aspartyl-type protease activity or the ability of CSP56 to permit metastases.

15 Antibodies which specifically bind to epitopes of metastatic marker proteins, polypeptides, fusion proteins, or biologically active variants can be used in immunochemical assays, including but not limited to Western Blots, ELISAs, radioimmunoassays, immunohistochemical assays, immunoprecipitations, or other
20 immunochemical assays known in the art. Typically, antibodies of the invention provide a detection signal at least 5-, 10-, or 20-fold higher than a detection signal provided with other proteins when used in such immunochemical assays. Preferably, antibodies which specifically bind to epitopes of a particular metastatic marker protein do not detect other proteins in immunochemical assays and can immunoprecipitate that metastatic marker protein or polypeptide fragments of the metastatic marker protein from solution.

25 Metastatic marker protein-specific antibodies specifically bind to epitopes present in a metastatic marker protein having an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence of SEQ ID NOS:1-18 or to biologically active variants of those amino acid sequences. Typically, at least 6, 8, 10, or 12 contiguous amino acids are required to form an epitope. However, epitopes which
30 involve non-contiguous amino acids may require more. *e.g.*, at least 15, 25, or 50 amino

acids. Preferably, metastatic marker protein epitopes are not present in other human proteins.

Epitopes of a metastatic marker protein which are particularly antigenic can be selected, for example, by routine screening of polypeptide fragments of the metastatic marker protein for antigenicity or by applying a theoretical method for selecting antigenic regions of a protein to the amino acid sequence of the metastatic marker protein. Such methods are taught, for example, in Hopp and Wood, *Proc. Natl. Acad. Sci. U.S.A.* 78, 3824-28 (1981), Hopp and Wood, *Mol. Immunol.* 20, 483-89 (1983), and Sutcliffe *et al.*, *Science* 219, 660-66 (1983). By reference to Figure 3, antigenic regions of CSP56 which could also bind to antibodies which crossreact with other aspartyl proteases can be avoided.

Any type of antibody known in the art can be generated to bind specifically to metastatic marker protein epitopes. For example, preparations of polyclonal and monoclonal antibodies can be made using standard methods which are well known in the art. Similarly, single-chain antibodies can also be prepared. Single-chain antibodies which specifically bind to metastatic marker protein epitopes can be isolated, for example, from single-chain immunoglobulin display libraries, as is known in the art. The library is "panned" against a metastatic marker protein amino acid sequence, and a number of single chain antibodies which bind with high-affinity to different epitopes of the metastatic marker protein can be isolated. Hayashi *et al.*, 1995, *Gene* 160:129-30. Single-chain antibodies can also be constructed using a DNA amplification method, such as the polymerase chain reaction (PCR), using hybridoma cDNA as a template. Thirion *et al.*, 1996, *Eur. J. Cancer Prev.* 5:507-11.

Single-chain antibodies can be mono- or bispecific, and can be bivalent or tetravalent. Construction of tetravalent, bispecific single-chain antibodies is taught, for example, in Coloma and Morrison, 1997, *Nat. Biotechnol.* 15:159-63. Construction of bivalent, bispecific single-chain antibodies is taught *inter alia* in Mallender and Voss, 1994, *J. Biol. Chem.* 269:199-206.

A nucleotide sequence encoding a single-chain antibody can be constructed using manual or automated nucleotide synthesis, cloned into an expression construct using

standard recombinant DNA methods, and introduced into a cell to express the coding sequence, as described below. Alternatively, single-chain antibodies can be produced directly using, for example, filamentous phage technology. Verhaar *et al.*, 1995, *Int. J. Cancer* 61:497-501; Nicholls *et al.*, 1993, *J. Immunol. Meth.* 165:81-91.

5 Monoclonal and other antibodies can also be "humanized" in order to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between, for example, rodent antibodies and human sequences can
10 be minimized by replacing residues which differ from those in the human sequences, for example, by site directed mutagenesis of individual residues, or by grating of entire complementarity determining regions. Alternatively, one can produce humanized antibodies using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to epitopes of a metastatic marker protein can contain antigen binding
15 sites which are either partially or fully humanized, as disclosed in U.S. 5,565,332.

Other types of antibodies can be constructed and used therapeutically in methods of the invention. For example, chimeric antibodies can be constructed as disclosed, for example, in WO 93/03151. Binding proteins which are derived from immunoglobulins and which are multivalent and multispecific, such as the "diabodies" described in WO
20 94/13804, can also be prepared.

Antibodies of the invention can be purified by methods well known in the art. For example, antibodies can be affinity purified by passing the antibodies over a column to which a metastatic marker protein, polypeptide, variant, or fusion protein is bound. The bound antibodies can then be eluted from the column, using a buffer with a high salt
25 concentration.

The invention also provides subgenomic polynucleotides which encode metastatic marker proteins, polypeptides, variants, or fusion proteins. Subgenomic polynucleotides contain less than a whole chromosome. Preferably, the subgenomic polynucleotides are intron-free. An isolated metastatic marker protein subgenomic
30 polynucleotide comprises at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30,

- 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2050, 2100, 2150, or 2200 contiguous nucleotides of SEQ ID NO:1; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, or 400
- 5 contiguous nucleotides of SEQ ID NOS:2 or 9; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2250, or 2500 contiguous nucleotides of SEQ ID NO:6; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, or 175 contiguous nucleotides of SEQ ID NO:7; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, or 350 contiguous nucleotides of SEQ ID NO:8; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, or 350 contiguous nucleotides of SEQ ID NO:12; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, or 300 contiguous nucleotides of SEQ ID NOS:3, 4, 5, or 10; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, or 500 contiguous nucleotides of SEQ ID NO:11; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, or 300 contiguous nucleotides of SEQ ID NO:13; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, or 950 contiguous nucleotides of SEQ ID NO:14; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, or 450 contiguous nucleotides of SEQ ID NO:15; at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75, 100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, 1850, 1900, 1950, 2000, 2250, 2500, 2750, 3000, 3250, or 3500 contiguous nucleotides of SEQ
- 25 ID NO:16; or at least 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, 50, 75,
- 30

100, 125, 150, 175, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, or 1500 contiguous nucleotides of SEQ ID NO:17 or can comprise one of SEQ ID NOS:1-17.

5 A *CSP56* polynucleotide can comprise a contiguous sequence of at least 10, 11, 12, 15, 20, 24, 25, 30, 32, 33, 35, 36, 40, 42, 45, 48, 50, 51, 54, 60, 63, 69, 70, 74, 75, 80, 84, 87, 90, 93, 96, 99, 100, 105, 114, 120, 125, 150, 225, 300, 333, 336, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1050, 1100, 1150, 1200, 1250, 1300, 1350, 1400, 1450, 1500, 1550, 1600, 1650, 1700, 1750, 1800, or 1850 nucleotides selected from SEQ ID NO:18 or can comprise SEQ ID NO:18. An isolated *CSP56* 10 polynucleotide encodes at least 8, 10, 12, 14, 15, 17, 18, 20, 25, 29, 30, 31, 32, 40, 50, 75, 100 or 111 contiguous amino acids of SEQ ID NO:19 and can encode the entire amino acid sequence shown in SEQ ID NO:19. Preferred *CSP56* polynucleotides encode at least amino acids 1-30, 8-20, 7-21, 6-22, 106-115, 105-116, 104-117, 100-120, 297-306, 296-307, 295-308, 290-320, 461-489, 460-490, 459-491, and 407-518 of SEQ ID 15 NO:19.

The complements of the nucleotide sequences shown in SEQ ID NOS:1-18 are contiguous nucleotide sequences which form Watson-Crick base pairs with a contiguous nucleotide sequence as shown in SEQ ID NOS:1-18. The complements of SEQ ID NOS:1-18 are also polynucleotides of the invention. Complements of coding sequences 20 can be used to provide antisense oligonucleotides and probes. Antisense oligonucleotides and probes of the invention can consist of at least 11, 12, 15, 20, 25, 30, 50, or 100 contiguous nucleotides. A complement of an entire coding sequence can also be used. Double-stranded polynucleotides which comprise all or a portion of the nucleotide sequences shown in SEQ ID NOS:1-18, as well as polynucleotides which 25 encode metastatic marker protein-specific antibodies or ribozymes, are also polynucleotides of the invention.

Degenerate nucleotide sequences encoding amino acid sequences of metastatic marker proteins and or variants, as well as homologous nucleotide sequences which are at least 65%, 75%, 85%, 90%, 95%, 98%, or 99% identical to the nucleotide sequences 30 shown in SEQ ID NOS:1-18, are also polynucleotides of the invention. Percent sequence

identity can be determined using computer programs which employ the Smith-Waterman algorithm, for example as implemented in the MPSRCH program (Oxford Molecular), using an affine gap search with the following parameters: a gap open penalty of 12 and a gap extension penalty of 1.

5 Typically, homologous polynucleotide sequences of the invention can be confirmed by hybridization under stringent conditions, as is known in the art. For example, using the following wash conditions--2 X SSC, 0.1% SDS, room temperature twice, 30 minutes each; then 2 X SSC, 0.1% SDS, 50 °C once for 30 minutes; then 2X SSC, room temperature twice, 10 minutes each-homologous sequences can be identified
10 that contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15%, 2-10%, or 1-5% basepair mismatches. Degrees of homology of polynucleotides of the invention can be selected by varying the stringency of the wash conditions for identification of clones from gene libraries (or other sources of genetic material), as is
15 well known in the art and described, for example, in manuals such as Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed. (1989).

Species homologs of subgenomic polynucleotides of the invention can also be identified by making suitable probes or primers and screening cDNA expression libraries or genomic libraries from other species, such as mice, monkeys, yeast, or bacteria.
20 Complete polynucleotide sequences can be obtained by chromosome walking, screening of libraries for overlapping clones, 5' RACE, or other techniques well known in the art. It is well known that the T_m of a double-stranded DNA decreases by 1-1.5 °C with every 1% decrease in homology (Bonner *et al.*, *J. Mol. Biol.* 81, 123 (1973). Homologous human polynucleotides or polynucleotides of other species can therefore be identified,
25 for example, by hybridizing a putative homologous polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NOS:1-18, comparing the melting temperature of the test hybrid with the melting temperature of a hybrid comprising a polynucleotide having a nucleotide sequence of SEQ ID NOS:1-18 and a polynucleotide which is perfectly complementary to the nucleotide sequence, and calculating the number of
30 basepair mismatches within the test hybrid.

Nucleotide sequences which hybridize to the nucleotide sequences shown in SEQ ID NOS:1-18 following stringent hybridization and/or wash conditions are also subgenomic polynucleotides of the invention. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook *et al.*, 1989, at
5 pages 9.50-9.51.

Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20 °C below the calculated T_m of the hybrid under study. The T_m of a hybrid between a polynucleotide sequence shown in SEQ ID NOS:1-18 and a polynucleotide sequence which is 65%,
10 75%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to that sequence can be calculated, for example, using the equation of Bolton and McCarthy, *Proc. Natl. Acad. Sci. U.S.A.* 48, 1390 (1962):

$$T_m = 81.5\text{ }^{\circ}\text{C} - 16.6(\log_{10}[\text{Na}^+]) + 0.41(\%G + C) - 0.63(\%\text{formamide}) - 600/l,$$

where l = the length of the hybrid in basepairs.

15 Stringent wash conditions include, for example, 4 X SSC at 65 °C, or 50% formamide, 4 X SSC at 42 °C, or 0.5 X SSC, 0.1% SDS at 65 °C. Highly stringent wash conditions include, for example, 0.2 X SSC at 65 °C.

Subgenomic polynucleotides can be purified free from other nucleotide sequences using standard nucleic acid purification techniques. For example, restriction enzymes
20 and probes can be used to isolate polynucleotides which comprise nucleotide sequences encoding metastatic marker proteins. Alternatively, PCR can be used to synthesize and amplify such polynucleotides. At least 90% of a preparation of isolated and purified polynucleotides comprises metastatic marker protein encoding polynucleotides.

Complementary DNA (cDNA) molecules which encode metastatic marker
25 proteins are also subgenomic polynucleotides of the invention. cDNA molecules can be made with standard molecular biology techniques, using mRNA as a template. cDNA molecules can thereafter be replicated using molecular biology techniques known in the art and disclosed in manuals such as Sambrook *et al.*, 1989. An amplification technique, such as the polymerase chain reaction (PCR), can be used to obtain additional copies of
30 subgenomic polynucleotides of the invention, using either human genomic DNA or

cDNA as a template.

Alternatively, synthetic chemistry techniques can be used to synthesize subgenomic polynucleotide molecules of the invention. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a metastatic marker protein having an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from SEQ ID NOS:1-17, a CSP56 amino acid sequence as shown in SEQ ID NO:19, or a biologically active variant of those sequences. All such nucleotide sequences are within the scope of the present invention.

The invention also provides polynucleotide probes which can be used to detect metastatic marker polypeptide sequences, for example, in hybridization protocols such as Northern or Southern blotting or *in situ* hybridizations. Polynucleotide probes of the invention comprise at least 12, 13, 14, 15, 16, 17, 18, 19, 20, 30, or 40 or more contiguous nucleotides selected from SEQ ID NOS:1-18. Polynucleotide probes of the invention can comprise a detectable label, such as a radioisotopic, fluorescent, enzymatic, or chemiluminescent label.

Isolated polynucleotides can be used, for example, as primers to obtain additional copies of the polynucleotides or as probes for detecting mRNA. Polynucleotides can also be used to express metastatic marker protein mRNA, protein, polypeptides, biologically active variants, single-chain antibodies, ribozymes, or fusion proteins.

Any of the polynucleotides described above can be present in a construct, such as a DNA or RNA construct. The construct can be a vector and can be used to transfer the polynucleotide into a cell, for example, for propagation of the polynucleotide. Constructs can be linear or circular molecules. They can be on autonomously replicating molecules or on molecules without replication sequences, and they can be regulated by their own or by other regulatory sequences, as is known in the art.

A construct can also be an expression construct. An expression construct comprises a promoter which is functional in a selected host cell. For example, the skilled artisan can readily select an appropriate promoter from the large number of cell type-specific promoters known and used in the art. The expression construct can also contain a transcription terminator which is functional in the host cell. The expression construct

comprises a polynucleotide segment which encodes, for example, all or a portion of a metastatic marker protein, polypeptide, biologically active variant, antibody, ribozyme, or fusion protein. The polynucleotide segment is located downstream from the promoter. Transcription of the polynucleotide segment initiates at the promoter. The expression
5 construct can be linear or circular and can contain sequences, if desired, for autonomous replication.

Subgenomic polynucleotides can be propagated in vectors and cell lines using techniques well known in the art. Expression systems in bacteria include those described in Chang *et al.*, *Nature* (1978) 275: 615, Goeddel *et al.*, *Nature* (1979) 281: 544,
10 Goeddel *et al.*, *Nucleic Acids Res.* (1980) 8: 4057, EP 36,776, U.S. 4,551,433, deBoer *et al.*, *Proc. Natl. Acad. Sci. USA* (1983) 80: 21-25, and Siebenlist *et al.*, *Cell* (1980) 20: 269.

Expression systems in yeast include those described in Hinnen *et al.*, *Proc. Natl. Acad. Sci. USA* (1978) 75: 1929; Ito *et al.*, *J. Bacteriol.* (1983) 153: 163; Kurtz *et al.*,
15 *Mol. Cell. Biol.* (1986) 6: 142; Kunze *et al.*, *J. Basic Microbiol.* (1985) 25: 141; Gleeson *et al.*, *J. Gen. Microbiol.* (1986) 132: 3459, Roggenkamp *et al.*, *Mol. Gen. Genet.* (1986) 202 :302) Das *et al.*, *J. Bacteriol.* (1984) 158: 1165; De Louvencourt *et al.*, *J. Bacteriol.* (1983) 154: 737, Van den Berg *et al.*, *Bio/Technology* (1990) 8: 135; Kunze *et al.*, *J. Basic Microbiol.* (1985) 25: 141; Cregg *et al.*, *Mol. Cell. Biol.* (1985) 5: 3376, U.S.
20 4,837,148, US 4,929,555; Beach and Nurse, *Nature* (1981) 300: 706; Davidow *et al.*, *Curr. Genet.* (1985) 10: 380, Gaillardin *et al.*, *Curr. Genet.* (1985) 10: 49, Ballance *et al.*, *Biochem. Biophys. Res. Commun.* (1983) 112: 284-289; Tilburn *et al.*, *Gene* (1983) 26: 205-221, Yelton *et al.*, *Proc. Natl. Acad. Sci. USA* (1984) 81: 1470-1474, Kelly and Hynes, *EMBO J.* (1985) 4: 475479; EP 244,234, and WO 91/00357.

25 Expression of subgenomic polynucleotides in insects can be accomplished as described in U.S. 4,745,051, Friesen *et al.* (1986) "The Regulation of Baculovirus Gene Expression" in: THE MOLECULAR BIOLOGY OF BACULOVIRUSES (W. Doerfler, ed.), EP 127,839, EP 155,476, and Vlak *et al.*, *J. Gen. Virol.* (1988) 69: 765-776, Miller *et al.*, *Ann. Rev. Microbiol.* (1988) 42: 177, Carbonell *et al.*, *Gene* (1988) 73: 409, Maeda *et al.*,
30 *Nature* (1985) 315: 592-594, Lebacq-Verheyden *et al.*, *Mol. Cell. Biol.* (1988) 8:

3129; Smith *et al.*, *Proc. Natl. Acad. Sci. USA* (1985) 82: 8404, Miyajima *et al.*, *Gene* (1987) 58: 273; and Martin *et al.*, *DNA* (1988) 7:99. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts are described in Luckow *et al.*, *Bio/Technology* (1988) 6: 47-55, Miller *et al.*, in GENETIC ENGINEERING (Setlow, J.K. *et al.* eds.), Vol. 8 (Plenum Publishing, 1986), pp. 277-279, and Maeda *et al.*, *Nature*, (1985) 315: 592-594.

Mammalian expression of subgenomic polynucleotides can be accomplished as described in Dijkema *et al.*, *EMBO J.* (1985) 4: 761, Gorman *et al.*, *Proc. Natl. Acad. Sci. USA* (1982b) 79: 6777, Boshart *et al.*, *Cell* (1985) 41: 521 and U.S. 4,399,216.

10 Other features of mammalian expression can be facilitated as described in Ham and Wallace, *Meth. Enz.* (1979) 58: 44, Barnes and Sato, *Anal. Biochem.* (1980) 102: 255, U.S. 4,767,704, US 4,657,866, US 4,927,762, US 4,560,655, WO 90/103430, WO 87/00195, and U.S. RE 30,985.

Subgenomic polynucleotides can be on linear or circular molecules. They can be

15 on autonomously replicating molecules or on molecules without replication sequences. They can be regulated by their own or by other regulatory sequences, as is known in the art. Subgenomic polynucleotides can be introduced into suitable host cells using a variety of techniques which are available in the art, such as transferrin-polycation-mediated DNA transfer, transfection with naked or encapsulated nucleic acids, liposome-mediated DNA transfer, intracellular transportation of DNA-coated latex beads,

20 protoplast fusion, viral infection, electroporation, and calcium phosphate-mediated transfection.

Polynucleotides of the invention can also be used in gene delivery vehicles, for the purpose of delivering an mRNA or oligonucleotide (either with the sequence of a

25 native mRNA or its complement), full-length protein, fusion protein, polypeptide, or ribozyme, or single-chain antibody, into a cell, preferably a eukaryotic cell. According to the present invention, a gene delivery vehicle can be, for example, naked plasmid DNA, a viral expression vector comprising a polynucleotide of the invention, or a polynucleotide of the invention in conjunction with a liposome or a condensing agent.

30 In one embodiment of the invention, the gene delivery vehicle comprises a

promoter and one of the polynucleotides disclosed herein. Preferred promoters are tissue-specific promoters and promoters which are activated by cellular proliferation, such as the thymidine kinase and thymidylate synthase promoters. Other preferred promoters include promoters which are activatable by infection with a virus, such as the
5 α - and β -interferon promoters, and promoters which are activatable by a hormone, such as estrogen. Other promoters which can be used include the Moloney virus LTR, the CMV promoter, and the mouse albumin promoter.

A gene delivery vehicle can comprise viral sequences such as a viral origin of replication or packaging signal. These viral sequences can be selected from viruses such
10 as astrovirus, coronavirus, orthomyxovirus, papovavirus, paramyxovirus, parvovirus, picornavirus, poxvirus, retrovirus, togavirus or adenovirus. In a preferred embodiment, the gene delivery vehicle is a recombinant retroviral vector. Recombinant retroviruses and various uses thereof have been described in numerous references including, for example, Mann *et al.*, *Cell* 33:153, 1983, Cane and Mulligan, *Proc. Nat'l. Acad. Sci. USA* 81:6349, 1984, Miller *et al.*, *Human Gene Therapy* 1:5-14, 1990, U.S. Patent Nos. 4,405,712, 4,861,719, and 4,980,289, and PCT Application Nos. WO 89/02,468, WO 89/05,349, and WO 90/02,806. Numerous retroviral gene delivery vehicles can be
15 utilized in the present invention, including for example those described in EP 0,415,731; WO 90/07936; WO 94/03622; WO 93/25698; WO 93/25234; U.S. Patent No. 5,219,740; WO 9311230; WO 9310218; Vile and Hart, *Cancer Res.* 53:3860-3864, 1993; Vile and Hart, *Cancer Res.* 53:962-967, 1993; Ram *et al.*, *Cancer Res.* 53:83-88, 1993; Takamiya *et al.*, *J. Neurosci. Res.* 33:493-503, 1992; Baba *et al.*, *J. Neurosurg.* 79:729-735, 1993 (U.S. Patent No. 4,777,127, GB 2,200,651, EP 0,345,242 and WO91/02805).

Particularly preferred retroviruses are derived from retroviruses which include
25 avian leukosis virus (ATCC Nos. VR-535 and VR-247), bovine leukemia virus (VR-1315), murine leukemia virus (MLV), mink-cell focus-inducing virus (Koch *et al.*, *J. Vir.* 49:828, 1984; and Oliff *et al.*, *J. Vir.* 48:542, 1983), murine sarcoma virus (ATCC Nos. VR-844, 45010 and 45016), reticuloendotheliosis virus (ATCC Nos VR-994, VR-770 and 45011), Rous sarcoma virus, Mason-Pfizer monkey virus, baboon endogenous virus,
30 endogenous feline retrovirus (*e.g.*, RD114), and mouse or rat gL30 sequences used as a

retroviral vector.

Particularly preferred strains of MLV from which recombinant retroviruses can be generated include 4070A and 1504A (Hartley and Rowe, *J. Vir.* 19:19, 1976), Abelson (ATCC No. VR-999), Friend (ATCC No. VR-245), Graffi (Ru *et al.*, *J. Vir.* 67:4722, 1993; and Yantchev *Neoplasma* 26:397, 1979), Gross (ATCC No. VR-590), Kirsten (Albino *et al.*, *J. Exp. Med.* 164:1710, 1986), Harvey sarcoma virus (Manly *et al.*, *J. Vir.* 62:3540, 1988; and Albino *et al.*, *J. Exp. Med.* 164:1710, 1986) and Rauscher (ATCC No. VR-998), and Moloney MLV (ATCC No. VR-190).

A particularly preferred non-mouse retrovirus is Rous sarcoma virus. Preferred Rous sarcoma viruses include Bratislava (Manly *et al.*, *J. Vir.* 62:3540, 1988; and Albino *et al.*, *J. Exp. Med.* 164:1710, 1986), Bryan high titer (*e.g.*, ATCC Nos. VR-334, VR-657, VR-726, VR-659, and VR-728), Bryan standard (ATCC No. VR-140), Carr-Zilber (Adighitov *et al.*, *Neoplasma* 27:159, 1980), Engelbreth-Holm (Laurent *et al.*, *Biochem Biophys Acta* 908:241, 1987), Harris, Prague (*e.g.*, ATCC Nos. VR-772, and 45033), and Schmidt-Ruppin (*e.g.* ATCC Nos. VR-724, VR-725, VR-354) viruses.

Any of the above retroviruses can be readily utilized in order to assemble or construct retroviral gene delivery vehicles given the disclosure provided herein and standard recombinant techniques (*e.g.*, Sambrook *et al.*, 1989, and Kunkle, *Proc. Natl. Acad. Sci. U.S.A.* 82:488, 1985) known in the art. Portions of retroviral expression vectors can be derived from different retroviruses. For example, retrovector LTRs can be derived from a murine sarcoma virus, a tRNA binding site from a Rous sarcoma virus, a packaging signal from a murine leukemia virus, and an origin of second strand synthesis from an avian leukosis virus. These recombinant retroviral vectors can be used to generate transduction competent retroviral vector particles by introducing them into appropriate packaging cell lines (*see* Serial No. 07/800,921, filed November 29, 1991). Recombinant retroviruses can be produced which direct the site-specific integration of the recombinant retroviral genome into specific regions of the host cell DNA. Such site-specific integration can be mediated by a chimeric integrase incorporated into the retroviral particle (*see* Serial No. 08/445,466 filed May 22, 1995). It is preferable that the recombinant viral gene delivery vehicle is a replication-defective recombinant virus.

Packaging cell lines suitable for use with the above-described retroviral gene delivery vehicles can be readily prepared (*see* Serial No. 08/240,030, filed May 9, 1994; *see also* WO 92/05266) and used to create producer cell lines (also termed vector cell lines or "VCLs") for production of recombinant viral particles. In particularly preferred
5 embodiments of the present invention, packaging cell lines are made from human (*e.g.*, HT1080 cells) or mink parent cell lines, thereby allowing production of recombinant retroviral gene delivery vehicles which are capable of surviving inactivation in human serum. The construction of recombinant retroviral gene delivery vehicles is described in detail in WO 91/02805. These recombinant retroviral gene delivery vehicles can be used
10 to generate transduction competent retroviral particles by introducing them into appropriate packaging cell lines (*see* Serial No. 07/800,921). Similarly, adenovirus gene delivery vehicles can also be readily prepared and utilized given the disclosure provided herein (*see also* Berkner, *Biotechniques* 6:616-627, 1988, and Rosenfeld *et al.*, *Science* 252:431-434, 1991, WO 93/07283, WO 93/06223, and WO 93/07282).

15 A gene delivery vehicle can also be a recombinant adenoviral gene delivery vehicle. Such vehicles can be readily prepared and utilized given the disclosure provided herein (*see* Berkner, *Biotechniques* 6:616, 1988, and Rosenfeld *et al.*, *Science* 252:431, 1991, WO 93/07283, WO 93/06223, and WO 93/07282). Adeno-associated viral gene delivery vehicles can also be constructed and used to deliver proteins or polynucleotides
20 of the invention to cells *in vitro* or *in vivo*. The use of adeno-associated viral gene delivery vehicles *in vitro* is described in Chatterjee *et al.*, *Science* 258: 1485-1488 (1992), Walsh *et al.*, *Proc. Nat'l. Acad. Sci.* 89: 7257-7261 (1992), Walsh *et al.*, *J. Clin. Invest.* 94: 1440-1448 (1994), Flotte *et al.*, *J. Biol. Chem.* 268: 3781-3790 (1993), Ponnazhagan *et al.*, *J. Exp. Med.* 179: 733-738 (1994), Miller *et al.*, *Proc. Nat'l Acad. Sci.* 91: 10183-10187 (1994), Einerhand *et al.*, *Gene Ther.* 2: 336-343 (1995), Luo *et al.*, *Exp. Hematol.* 23: 1261-1267 (1995), and Zhou *et al.*, *Gene Therapy* 3: 223-229 (1996). *In vivo* use of these vehicles is described in Flotte *et al.*, *Proc. Nat'l Acad. Sci.* 90: 10613-10617 (1993), and Kaplitt *et al.*, *Nature Genet.* 8:148-153 (1994).

30 In another embodiment of the invention, a gene delivery vehicle is derived from a togavirus. Preferred togaviruses include alphaviruses, in particular those described in

U.S. Serial No. 08/405,627, filed March 15, 1995, WO 95/07994. Alpha viruses, including Sindbis and ELVS viruses can be gene delivery vehicles for polynucleotides of the invention. Alpha viruses are described in WO 94/21792, WO 92/10578 and WO 95/07994. Several different alphavirus gene delivery vehicle systems can be constructed
5 and used to deliver polynucleotides to a cell according to the present invention. Representative examples of such systems include those described in U.S. Patents 5,091,309 and 5,217,879. Particularly preferred alphavirus gene delivery vehicles for use in the present invention include those which are described in WO 95/07994, and U.S. Serial No. 08/405,627.

10 Preferably, the recombinant viral vehicle is a recombinant alphavirus viral vehicle based on a Sindbis virus. Sindbis constructs, as well as numerous similar constructs, can be readily prepared essentially as described in U.S. Serial No. 08/198,450. Sindbis viral gene delivery vehicles typically comprise a 5' sequence capable of initiating Sindbis virus transcription, a nucleotide sequence encoding Sindbis non-structural proteins, a
15 viral junction region inactivated so as to prevent fragment transcription, and a Sindbis RNA polymerase recognition sequence. Optionally, the viral junction region can be modified so that polynucleotide transcription is reduced, increased, or maintained. As will be appreciated by those in the art, corresponding regions from other alphaviruses can be used in place of those described above.

20 The viral junction region of an alphavirus-derived gene delivery vehicle can comprise a first viral junction region which has been inactivated in order to prevent transcription of the polynucleotide and a second viral junction region which has been modified such that polynucleotide transcription is reduced. An alphavirus-derived vehicle can also include a 5' promoter capable of initiating synthesis of viral RNA from
25 cDNA and a 3' sequence which controls transcription termination.

Other recombinant togaviral gene delivery vehicles which can be utilized in the present invention include those derived from Semliki Forest virus (ATCC VR-67; ATCC VR-1247), Middleberg virus (ATCC VR-370), Ross River virus (ATCC VR-373; ATCC VR-1246), Venezuelan equine encephalitis virus (ATCC VR923; ATCC VR-1250;
30 ATCC VR-1249; ATCC VR-532), and those described in U.S. Patents 5,091,309 and

5,217,879 and in WO 92/10578. The Sindbis vehicles described above, as well as numerous similar constructs, can be readily prepared essentially as described in U.S. Serial No. 08/198,450.

Other viral gene delivery vehicles suitable for use in the present invention include, for example, those derived from poliovirus (Evans *et al.*, *Nature* 339:385, 1989, and Sabin *et al.*, *J. Biol. Standardization* 1:115, 1973) (ATCC VR-58); rhinovirus (Arnold *et al.*, *J. Cell. Biochem.* L401, 1990) (ATCC VR-1110); pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch *et al.*, *PROC. NATL. ACAD. SCI. U.S.A.* 86:317, 1989; Flexner *et al.*, *Ann. N.Y. Acad. Sci.* 569:86, 1989; Flexner *et al.*, *Vaccine* 8:17, 1990; U.S. 4,603,112 and U.S. 4,769,330; WO 89/01973) (ATCC VR-111; ATCC VR-2010); SV40 (Mulligan *et al.*, *Nature* 277:108, 1979) (ATCC VR-305), (Madzak *et al.*, *J. Gen. Vir.* 73:1533, 1992); influenza virus (Luytjes *et al.*, *Cell* 59:1107, 1989; McMichael *et al.*, *The New England Journal of Medicine* 309:13, 1983; and Yap *et al.*, *Nature* 273:238, 1978) (ATCC VR-797); parvovirus such as adeno-associated virus (Samulski *et al.*, *J. Vir.* 63:3822, 1989, and Mendelson *et al.*, *Virology* 166:154, 1988) (ATCC VR-645); herpes simplex virus (Kit *et al.*, *Adv. Exp. Med. Biol.* 215:219, 1989) (ATCC VR-977; ATCC VR-260); *Nature* 277: 108, 1979); human immunodeficiency virus (EPO 386,882, Buchschacher *et al.*, *J. Vir.* 66:2731, 1992); measles virus (EPO 440,219) (ATCC VR-24); A (ATCC VR-67; ATCC VR-1247), Aura (ATCC VR-368), Bebaru virus (ATCC VR-600; ATCC VR-1240), Cabassou (ATCC VR-922), Chikungunya virus (ATCC VR-64; ATCC VR-1241), Fort Morgan (ATCC VR-924), Getah virus (ATCC VR-369; ATCC VR-1243), Kyzylagach (ATCC VR-927), Mayaro (ATCC VR-66), Mucambo virus (ATCC VR-580; ATCC VR-1244), Ndumu (ATCC VR-371), Pixuna virus (ATCC VR-372; ATCC VR-1245), Tonate (ATCC VR-925), Trinita (ATCC VR-469), Una (ATCC VR-374), Whataroa (ATCC VR-926), Y-62-33 (ATCC VR-375), O'Nyong virus, Eastern encephalitis virus (ATCC VR-65; ATCC VR-1242), Western encephalitis virus (ATCC VR-70; ATCC VR-1251; ATCC VR-622; ATCC VR-1252), and coronavirus (Hamre *et al.*, *Proc. Soc. Exp. Biol. Med.* 121:190, 1966) (ATCC VR-740).

30 A polynucleotide of the invention can also be combined with a condensing agent

to form a gene delivery vehicle. In a preferred embodiment, the condensing agent is a polycation, such as polylysine, polyarginine, polyornithine, protamine, spermine, spermidine, and putrescine. Many suitable methods for making such linkages are known in the art (see, for example, Serial No. 08/366,787, filed December 30, 1994).

5 In an alternative embodiment, a polynucleotide is associated with a liposome to form a gene delivery vehicle. Liposomes are small, lipid vesicles comprised of an aqueous compartment enclosed by a lipid bilayer, typically spherical or slightly elongated structures several hundred Angstroms in diameter. Under appropriate conditions, a liposome can fuse with the plasma membrane of a cell or with the
10 membrane of an endocytic vesicle within a cell which has internalized the liposome, thereby releasing its contents into the cytoplasm. Prior to interaction with the surface of a cell, however, the liposome membrane acts as a relatively impermeable barrier which sequesters and protects its contents, for example, from degradative enzymes.

Because a liposome is a synthetic structure, specially designed liposomes can be
15 produced which incorporate desirable features. See Stryer, *Biochemistry*, pp. 236-240, 1975 (W.H. Freeman, San Francisco, CA); Szoka *et al.*, *Biochim. Biophys. Acta* 600:1, 1980; Bayer *et al.*, *Biochim. Biophys. Acta* 550:464, 1979; Rivnay *et al.*, *Meth. Enzymol.* 149:119, 1987; Wang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 84: 7851, 1987, Plant *et al.*, *Anal. Biochem.* 176:420, 1989, and U.S. Patent 4,762,915. Liposomes can encapsulate a
20 variety of nucleic acid molecules including DNA, RNA, plasmids, and expression constructs comprising polynucleotides such those disclosed in the present invention.

Liposomal preparations for use in the present invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7413-7416, 1987), mRNA (Malone *et al.*, *Proc. Natl. Acad. Sci. USA* 86:6077-6081, 1989), and purified transcription factors (Debs *et al.*, *J. Biol. Chem.* 265:10189-10192, 1990), in functional form. Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are available under the trademark Lipofectin, from GIBCO BRL,
30 Grand Island, NY. See also Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 5148-5152.87,

1994. Other commercially available liposomes include Transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer). Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. *See, e.g., Szoka et al., Proc. Natl. Acad. Sci. USA 75:4194-4198, 1978; and WO 90/11092 for descriptions of the*
5 *synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes.*

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, AL), or can be easily prepared using readily available materials. Such materials include phosphatidyl choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol
10 (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

The liposomes can comprise multilammellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs). The various liposome-nucleic
15 acid complexes are prepared using methods known in the art. *See, e.g., Straubinger et al., METHODS OF IMMUNOLOGY (1983), Vol. 101, pp. 512-527; Szoka et al., Proc. Natl. Acad. Sci. USA 87:3410-3414, 1990; Papahadjopoulos et al., Biochim. Biophys. Acta 394:483, 1975; Wilson et al., Cell 17:77, 1979; Deamer and Bangham, Biochim. Biophys. Acta 443:629, 1976; Ostro et al., Biochem. Biophys. Res. Commun. 76:836 ,*
20 *1977; Fraley et al., Proc. Natl. Acad. Sci. USA 76:3348, 1979; Enoch and Strittmatter, Proc. Natl. Acad. Sci. USA 76:145, 1979; Fraley et al., J. Biol. Chem. 255:10431, 1980; Szoka and Papahadjopoulos, Proc. Natl. Acad. Sci. USA 75:145, 1979; and Schaefer-Ridder et al., Science 215:166, 1982.*

In addition, lipoproteins can be included with a polynucleotide of the invention
25 for delivery to a cell. Examples of such lipoproteins include chylomicrons, HDL, IDL, LDL, and VLDL. Mutants, fragments, or fusions of these proteins can also be used. Modifications of naturally occurring lipoproteins can also be used, such as acetylated LDL. These lipoproteins can target the delivery of polynucleotides to cells expressing lipoprotein receptors. Preferably, if lipoproteins are included with a polynucleotide, no
30 other targeting ligand is included in the composition.

In another embodiment, naked polynucleotide molecules are used as gene delivery vehicles, as described in WO 90/11092 and U.S. Patent 5,580,859. Such gene delivery vehicles can be either DNA or RNA and, in certain embodiments, are linked to killed adenovirus. Curiel *et al.*, *Hum. Gene. Ther.* 3:147-154, 1992. Other suitable
5 vehicles include DNA-ligand (Wu *et al.*, *J. Biol. Chem.* 264:16985-16987, 1989), lipid-DNA combinations (Felgner *et al.*, *Proc. Natl. Acad. Sci. USA* 84:7413-7417, 1989), liposomes (Wang *et al.*, *Proc. Natl. Acad. Sci.* 84:7851-7855, 1987) and microprojectiles (Williams *et al.*, *Proc. Natl. Acad. Sci.* 88:2726-2730, 1991).

One can increase the efficiency of naked polynucleotide uptake into cells by
10 coating the polynucleotides onto biodegradable latex beads. This approach takes advantage of the observation that latex beads, when incubated with cells in culture, are efficiently transported and concentrated in the perinuclear region of the cells. The beads will then be transported into cells when injected into muscle. Polynucleotide-coated latex beads will be efficiently transported into cells after endocytosis is initiated by the
15 latex beads and thus increase gene transfer and expression efficiency. This method can be improved further by treating the beads to increase their hydrophobicity, thereby facilitating the disruption of the endosome and release of polynucleotides into the cytoplasm.

The invention also provides a method of detecting metastatic marker genes
20 expression in a biological sample, such as a tissue sample of the breast or colon. Detection of metastatic marker genes expression is useful, for example, for identifying metastatic tissue and identifying metastatic potential of a tissue, to identify patients who are at risk for developing metastatic cancers in other organs of the body.

The tissue sample can be, for example, a solid tissue or a fluid sample. Protein or
25 nucleic acid expression products can be detected in the tissue sample. In one embodiment, the tissue sample is assayed for the presence of a metastatic marker proteins. The metastatic marker protein has a sequence encoded by polynucleotides comprising SEQ ID NOS:1-18 and can be detected using the metastatic marker protein-specific antibodies of the present invention. The antibodies can be labeled, for example,
30 with a radioactive, fluorescent, biotinylated, or enzymatic tag and detected directly, or

can be detected using indirect immunochemical methods, using a labeled secondary antibody. The presence of the metastatic marker proteins can be assayed, for example, in tissue sections by immunocytochemistry, or in lysates, using Western blotting, as is known in the art.

5 In another embodiment, the tissue sample is assayed for the presence of metastatic marker protein mRNA. Metastatic marker protein mRNA can be detected by *in situ* hybridization in tissue sections or in Northern blots containing poly A+ mRNA. Metastatic marker protein-specific probes may be generated using the cDNA sequences disclosed in SEQ ID NOS:1-18. The probes are preferably 15 to 50 nucleotides in
10 length, although they may be 8, 10, 11, 12, 20, 25, 30, 35, 40, 45, 60, 75, or 100 nucleotides in length. The probes can be synthesized chemically or can be generated from longer polynucleotides using restriction enzymes. The probes can be labeled, for example, with a radioactive, biotinylated, or fluorescent tag. If desired, the tissue sample can be subjected to a nucleic acid amplification process.

15 A tissue sample in which an expression product of a polynucleotide comprising SEQ ID NOS:1, 4, 11, 16, 17, or 18 is detected is identified as metastatic or as having metastatic potential. A tissue sample in which an expression product of a polynucleotide comprising SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, or 15 is identified as not metastatic or as having a low metastatic potential.

20 Propensity for high- or low-grade metastasis of a colon tumor can also be predicted, by measuring in a colon tumor sample an expression product of a gene comprising the nucleotide sequence of SEQ ID NOS:16 or 17. A colon tumor sample which expresses a product of a gene comprising the nucleotide sequence of SEQ ID NO:16 is categorized as having a high propensity to metastasize. A colon tumor sample
25 which expresses a product of a gene comprising the nucleotide sequence of SEQ ID NO:17 is categorized as having a low propensity to metastasize.

 Optionally, the level of a particular metastatic marker expression product in a tissue sample can be quantitated. Quantitation can be accomplished, for example, by comparing the level of expression product detected in the tissue sample with the amounts
30 of product present in a standard curve. A comparison can be made visually or using a

technique such as densitometry, with or without computerized assistance. For use as controls, tissue samples can be isolated from other humans, other non-cancerous organs of the patient being tested, or preferably non-metastatic breast or colon cancer from the patient being tested.

5 Polynucleotides encoding metastatic marker-specific reagents of the invention, such as antibodies and nucleotide probes, can be supplied in a kit for detecting them in a biological sample. The kit can also contain buffers or labeling components, as well as instructions for using the reagents to detect the metastatic marker expression products in the biological sample.

10 Metastatic marker gene expression in a cell can be increased or decreased, as desired. Metastatic marker genes expression can be altered for therapeutic purposes, as described below, or can be used to identify therapeutic agents.

In one embodiment of the invention, expression of a metastatic marker gene whose expression is upregulated in metastatic cancer is decreased using a ribozyme, an RNA molecule with catalytic activity. *See, e.g., Cech, 1987, Science 236: 1532-1539; Cech, 1990, Ann. Rev. Biochem. 59:543-568; Cech, 1992, Curr. Opin. Struct. Biol. 2: 605-609; Couture and Stinchcomb, 1996, Trends Genet. 12: 510-515.* Ribozymes can be used to inhibit gene function by cleaving an RNA sequence, as is known in the art (*e.g., Haseloff et al., U.S. 5,641,673*).

20 The coding sequence of the metastatic marker genes can be used to generate a ribozyme which will specifically bind to mRNA transcribed from a metastatic marker genes. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (*see Haseloff et al. (1988), Nature 334:585-591*). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (*see, for example, Gerlach et al., EP 321,201*). Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related; thus, upon

25
30

hybridizing to the target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Ribozymes can be introduced into cells as part of a DNA construct, as is known in the art. The DNA construct can also include transcriptional regulatory elements, such as a promoter element, an enhancer or UAS element, and a transcriptional terminator signal, for controlling the transcription of the ribozyme in the cells.

Mechanical methods, such as microinjection, liposome-mediated transfection, electroporation, or calcium phosphate precipitation, can be used to introduce the ribozyme-containing DNA construct into cells whose division it is desired to decrease, as described above. Alternatively, if it is desired that the DNA construct be stably retained by the cells, the DNA construct can be supplied on a plasmid and maintained as a separate element or integrated into the genome of the cells, as is known in the art.

As taught in Haseloff *et al.*, U.S. 5,641,673, the ribozyme can be engineered so that its expression will occur in response to factors which induce expression of the metastatic marker genes. The ribozyme can also be engineered to provide an additional level of regulation, so that destruction of mRNA occurs only when both the ribozyme and the metastatic marker genes are induced in the cells.

Expression of the metastatic marker genes can also be altered using an antisense oligonucleotide sequence. The antisense sequence is complementary to at least a portion of the coding sequence of a metastatic marker genes having the nucleotide sequence shown in SEQ ID NO: 1-18. The complement of the nucleotide sequence shown in SEQ ID NO:1-18 consists of a contiguous sequence of nucleotides which form Watson-Crick basepairs with the contiguous nucleotide sequence shown in SEQ ID NO:1-18.

Preferably, the antisense oligonucleotide sequence is at least six nucleotides in length, but can be about 8, 12, 15, 20, 25, 30, 35, 40, 45, or 50 nucleotides long. Longer sequences can also be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into cells whose division is to be decreased, as described above.

Antisense oligonucleotides can be composed of deoxyribonucleotides, ribonucleotides, or a combination of both. Oligonucleotides can be synthesized manually

or by an automated synthesizer, by covalently linking the 5' end of one nucleotide with the 3' end of another nucleotide with non-phosphodiester internucleotide linkages such as alkylphosphonates, phosphorothioates, phosphorodithioates, alkylphosphonothioates, alkylphosphonates, phosphoramidates, phosphate esters, carbamates, acetamidate, carboxymethyl esters, carbonates, and phosphate triesters. See Brown, 1994, *Meth. Mol. Biol.* 20:1-8; Sonveaux, 1994, *Meth. Mol. Biol.* 26:1-72; Uhlmann *et al.*, 1990, *Chem. Rev.* 90:543-583.

Precise complementarity is not required for successful duplex formation between an antisense molecule and the complementary coding sequence of a metastatic marker gene. Antisense molecules which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a portion of a coding sequence of a metastatic marker gene, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent coding sequences, can provide targeting specificity for mRNA of a metastatic marker gene. Preferably, each stretch of contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular metastatic marker gene coding sequence.

Antisense oligonucleotides can be modified without affecting their ability to hybridize to a metastatic marker protein coding sequence. These modifications can be internal or at one or both ends of the antisense molecule. For example, internucleoside phosphate linkages can be modified by adding cholesteryl or diamine moieties with varying numbers of carbon residues between the amino groups and terminal ribose. Modified bases and/or sugars, such as arabinose instead of ribose, or a 3', 5'-substituted oligonucleotide in which the 3' hydroxyl group or the 5' phosphate group are substituted, can also be employed in a modified antisense oligonucleotide. These modified oligonucleotides can be prepared by methods well known in the art. Agrawal *et al.*, 1992, *Trends Biotechnol.* 10:152-158; Uhlmann *et al.*, 1990, *Chem. Rev.* 90:543-584; Uhlmann *et al.*, 1987, *Tetrahedron. Lett.* 215:3539-3542.

Antibodies of the invention which specifically bind to a metastatic marker protein can also be used to alter metastatic marker gene expression. Specific antibodies bind to the metastatic marker proteins and prevent the protein from functioning in the cell. Polynucleotides encoding specific antibodies of the invention can be introduced into
5 cells, as described above.

To increase expression of metastatic marker genes which are down-regulated in metastatic cells, all or a portion of a metastatic marker gene or expression product can be introduced into a cell. Optionally, the gene or expression product can be a component of a therapeutic composition comprising a pharmaceutically acceptable carrier (see below).
10 The entire coding sequence can be introduced, as described above. Alternatively, a portion of the metastatic marker protein or a nucleotide sequence encoding it can be introduced into the cell.

Expression of an endogenous metastatic marker genes in a cell can also be altered by introducing in frame with the endogenous metastatic marker genes a DNA construct
15 comprising a metastatic marker protein targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site by homologous recombination, such that a homologously recombinant cell comprising the DNA construct is formed. The new transcription unit can be used to turn the metastatic marker genes on or off as desired. This method of affecting endogenous gene expression is taught in U.S. Patent No.
20 5,641,670.

The targeting sequence is a segment of at least 10, 12, 15, 20, or 50 contiguous nucleotides selected from the nucleotide sequence shown in SEQ ID NO:1-18. The transcription unit is located upstream of a coding sequence of the endogenous metastatic marker protein gene. The exogenous regulatory sequence directs transcription of the
25 coding sequence of the metastatic marker genes.

Expression of the metastatic marker proteins of the present invention can be used to screen for drugs which have a therapeutic anti-metastatic effect. The effect of a test compound on metastatic marker protein synthesis can also be used to identify test compounds which modulate metastasis. Synthesis of metastatic marker proteins in a
30 biological sample, such as a cell culture, tissue sample, or cell-free homogenate, can be

measured by any means for measuring protein synthesis known in the art, such as incorporation of labeled amino acids into proteins and detection of labeled metastatic marker proteins in a polyacrylamide gel. The amount of metastatic marker proteins can be detected, for example, using metastatic marker protein-specific antibodies of the invention in Western blots. The amount of the metastatic marker proteins synthesized in the presence or absence of a test compound can be determined by any means known in the art, such as comparison of the amount of metastatic marker protein synthesized with the amount of the metastatic marker proteins present in a standard curve.

The effect of a test compound on metastatic marker protein synthesis can also be measured by Northern blot analysis, by measuring the amount of metastatic marker protein mRNA expression in response to the test compound using metastatic marker protein specific nucleotide probes of the invention, as is known in the art. A test compound which decreases synthesis of a metastatic marker protein encoded by a polynucleotide comprising SEQ ID NOS:1, 4, 11, 16, 17, or 18 or which increases synthesis of a metastatic marker protein encoded by a polynucleotide comprising SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, or 15 is identified as a possible therapeutic agent.

Typically, a biological sample, such as a breast or colon sample, is contacted with a range of concentrations of the test compound, such as 1.0 nM, 5.0 nM, 10 nM, 50 nM, 100 nM, 500 nM, 1 mM, 10 mM, 50 mM, and 100 mM. Preferably, the test compound increases or decreases expression of a metastatic marker protein by 60%, 75%, or 80%. More preferably, an increase or decrease of 85%, 90%, 95%, or 98% is achieved.

The invention provides therapeutic compositions for increasing or decreasing expression of metastatic marker protein as is appropriate. Therapeutic compositions for increasing metastatic marker gene expression are desirable for metastatic markers down-regulated in metastatic cells. These comprise polynucleotides encoding all or a portion of a metastatic marker protein gene expression product. Preferably, the therapeutic composition contains an expression construct comprising a promoter and a polynucleotide segment encoding at least six contiguous amino acids of the metastatic marker protein. Within the expression construct, the polynucleotide segment is located downstream from the promoter, and transcription of the polynucleotide segment initiates

at the promoter. A more complete description of gene transfer vectors, especially retroviral vectors is contained in U.S. Serial No. 08/869,309.

Decreased metastatic marker gene expression is desired in conditions in which the metastatic marker gene is upregulated in metastatic cancer. Therapeutic
5 compositions for treating these disorders comprise a polynucleotide encoding a reagent which specifically binds to a metastatic marker protein expression product, as disclosed herein.

Metastatic marker therapeutic compositions of the invention also comprise a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are well
10 known to those in the art. Such carriers include, but are not limited to, large, slowly metabolized macromolecules, such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles. Pharmaceutically acceptable salts can also be used in the composition, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as
15 well as the salts of organic acids such as acetates, propionates, malonates, or benzoates.

Therapeutic compositions can also contain liquids, such as water, saline, glycerol, and ethanol, as well as substances such as wetting agents, emulsifying agents, or pH buffering agents. Liposomes, such as those described in U.S. 5,422,120, WO 95/13796, WO 91/14445, or EP 524,968 B1, can also be used as a carrier for the therapeutic
20 composition.

Typically, a therapeutic metastatic marker composition is prepared as an injectable, either as a liquid solution or suspension; however, solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. A metastatic marker composition can also be formulated into an enteric coated tablet or gel
25 capsule according to known methods in the art, such as those described in U.S. 4,853,230, EP 225,189, AU 9,224,296, and AU 9,230,801.

Administration of the metastatic marker therapeutic agents of the invention can include local or systemic administration, including injection, oral administration, particle gun, or catheterized administration, and topical administration. Various methods can be
30 used to administer a therapeutic metastatic marker composition directly to a specific site

in the body.

For treatment of tumors, for example, a small tumor or metastatic lesion can be located and a therapeutic metastatic marker composition injected several times in several different locations within the body of tumor. Alternatively, arteries which serve a tumor
5 can be identified, and a therapeutic composition injected into such an artery, in order to deliver the composition directly into the tumor.

A tumor which has a necrotic center can be aspirated and the composition injected directly into the now empty center of the tumor. A therapeutic metastatic marker composition can be directly administered to the surface of a tumor, for example,
10 by topical application of the composition. X-ray imaging can be used to assist in certain of the above delivery methods. Combination therapeutic agents, including an the metastatic marker protein, polypeptide, or subgenomic polynucleotide and other therapeutic agents, can be administered simultaneously or sequentially.

Receptor-mediated targeted delivery can be used to deliver therapeutic
15 compositions containing subgenomic polynucleotides, proteins, or reagents such as antibodies, ribozymes, or antisense oligonucleotides to specific tissues. Receptor-mediated delivery techniques are described in, for example, Findeis *et al.* (1993), *Trends in Biotechnol.* 11, 202-05; Chiou *et al.* (1994), GENE THERAPEUTICS: METHODS AND APPLICATIONS OF DIRECT GENE TRANSFER (J.A. Wolff, ed.); Wu & Wu (1988), *J. Biol.*
20 *Chem.* 263, 621-24; Wu *et al.* (1994), *J. Biol. Chem.* 269, 542-46; Zenke *et al.* (1990), *Proc. Natl. Acad. Sci. U.S.A.* 87, 3655-59; Wu *et al.* (1991), *J. Biol. Chem.* 266, 338-42.

Alternatively, a metastatic marker therapeutic composition can be introduced into human cells *ex vivo*, and the cells then replaced into the human. Cells can be removed from a variety of locations including, for example, from a selected tumor or from an
25 affected organ. In addition, a therapeutic composition can be inserted into non-affected, for example, dermal fibroblasts or peripheral blood leukocytes. If desired, particular fractions of cells such as a T cell subset or stem cells can also be specifically removed from the blood (*see*, for example, PCT WO 91/16116). The removed cells can then be contacted with a metastatic marker therapeutic composition utilizing any of the above-
30 described techniques, followed by the return of the cells to the human, preferably to or

within the vicinity of a tumor or other site to be treated. The methods described above can additionally comprise the steps of depleting fibroblasts or other non-contaminating tumor cells subsequent to removing tumor cells from a human, and/or the step of inactivating the cells, for example, by irradiation.

5 Both the dose of a metastatic marker composition and the means of administration can be determined based on the specific qualities of the therapeutic composition, the condition, age, and weight of the patient, the progression of the disease, and other relevant factors. Preferably, a therapeutic composition of the invention increases or decreases expression of the metastatic marker genes by 50%, 60%, 70%, or
10 80%. Most preferably, expression of the metastatic marker genes is increased or decreased by 90%, 95%, 99%, or 100%. The effectiveness of the mechanism chosen to alter expression of the metastatic marker genes can be assessed using methods well known in the art, such as hybridization of nucleotide probes to mRNA of the metastatic marker genes, quantitative RT-PCR, or detection of metastatic marker proteins using
15 specific antibodies.

 If the composition contains the metastatic marker proteins, polypeptide, or antibody, effective dosages of the composition are in the range of about 5 μ g to about 50 μ g/kg of patient body weight, about 50 μ g to about 5 mg/kg, about 100 μ g to about 500 μ g/kg of patient body weight, and about 200 to about 250 μ g/kg.

20 Therapeutic compositions containing metastatic marker subgenomic polynucleotides can be administered in a range of about 100 ng to about 200 mg of DNA for local administration in a gene therapy protocol. Concentration ranges of about 500 ng to about 50 mg, about 1 μ g to about 2 mg, about 5 μ g to about 500 μ g, and about 20 μ g to about 100 μ g of DNA can also be used during a gene therapy protocol. Factors
25 such as method of action and efficacy of transformation and expression are considerations that will affect the dosage required for ultimate efficacy of the metastatic marker protein subgenomic polynucleotides. Where greater expression is desired over a larger area of tissue, larger amounts of metastatic marker protein subgenomic polynucleotides or the same amounts readministered in a successive protocol of
30 administrations, or several administrations to different adjacent or close tissue portions

of, for example, a tumor site, may be required to effect a positive therapeutic outcome. In all cases, routine experimentation in clinical trials will determine specific ranges for optimal therapeutic effect.

Metastatic marker subgenomic polynucleotides of the invention can also be used
5 on polynucleotide arrays. Polynucleotide arrays provide a high throughput technique that can assay a large number of polynucleotide sequences in a single sample. This technology can be used, for example, as a diagnostic tool to identify metastatic lesions or to assess the metastatic potential of a tumor.

To create arrays, single-stranded polynucleotide probes can be spotted onto a
10 substrate in a two-dimensional matrix or array. Each single-stranded polynucleotide probe can comprise at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 or more contiguous nucleotides selected from the nucleotide sequences shown in SEQ ID NOS:1-18. Preferred arrays comprise at least one single-stranded polynucleotide probe comprising at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 or more
15 contiguous nucleotides selected from the nucleotide sequences shown in SEQ ID NOS:1, 4, 11, 16, 17, and 18. Other preferred arrays comprise at least one single-stranded polynucleotide probe comprising at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 or more contiguous nucleotides selected from the nucleotide sequences shown in SEQ ID NOS:2, 3, 6, 7, 9, 10, 12, 13, and 15. Still other preferred arrays
20 comprise at least one single-stranded polynucleotide probe comprising at least 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, or 30 or more contiguous nucleotides selected from the nucleotide sequences shown in SEQ ID NOS: 5 and 14 or SEQ ID NOS:16 and 17.

The substrate can be any substrate to which polynucleotide probes can be
25 attached, including but not limited to glass, nitrocellulose, silicon, and nylon. Polynucleotide probes can be bound to the substrate by either covalent bonds or by non-specific interactions, such as hydrophobic interactions. Techniques for constructing arrays and methods of using these arrays are described in EP No. 0 799 897; PCT No. WO 97/29212; PCT No. WO 97/27317; EP No. 0 785 280; PCT No. WO 97/02357; U.S.
30 Pat. No. 5,593,839; U.S. Pat. No. 5,578,832; EP No. 0 728 520; U.S. Pat. No. 5,599,695;

EP No. 0 721 016; U.S. Pat. No. 5,556,752; PCT No. WO 95/22058; and U.S. Pat. No. 5,631,734. Commercially available polynucleotide arrays, such as Affymetrix GeneChip[®], can also be used. Use of the GeneChip[®] to detect gene expression is described, for example, in Lockhart *et al.*, *Nature Biotechnology* 14:1675 (1996); Chee *et al.*, *Science* 274:610 (1996); Hacia *et al.*, *Nature Genetics* 14:441, 1996; and Kozal *et al.*, *Nature Medicine* 2:753, 1996.

Tissue samples which are suspected of being metastatic or the metastatic potential of which is unknown can be treated to form single-stranded polynucleotides, for example by heating or by chemical denaturation, as is known in the art. The single-stranded polynucleotides in the tissue sample can then be labeled and hybridized to the polynucleotide probes on the array. Detectable labels which can be used include but are not limited to radiolabels, biotinylated labels, fluorophors, and chemiluminescent labels. Double stranded polynucleotides, comprising the labeled sample polynucleotides bound to polynucleotide probes, can be detected once the unbound portion of the sample is washed away. Detection can be visual or with computer assistance.

Detection of a double-stranded polynucleotide comprising contiguous nucleotides selected from the group consisting of SEQ ID NOS:1-4, 11, 16, 17, and 18 or lack of detection of a double-stranded polynucleotide comprising contiguous nucleotides selected from the group consisting of SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, and 15 identifies the tissue sample as metastatic or of having metastatic potential.

All of the references cited in this disclosure are expressly incorporated herein by reference. The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.

EXPERIMENTAL PROCEDURES

The following materials and methods were used in the examples below.

Cell lines. Cell lines MCF-7, BR-3, BT-20, ZR-75-1, MDA-MB-157, MDA-MB-231, MDA-MB-361, MDA-MB-435, MDA-MB-453, MDA-MB-468, Alab, and
5 Hs578Bst were obtained from American Type Culture Collection. All cell lines were grown according to their specifications.

Differential Display. Differential display was performed using the Hieroglyph mRNA profile kit according to the manufacturer's directions (Genomix Corp., Foster City, CA). A total of 200 primer pairs were used to profile gene expression. Following
10 amplification of randomly primed mRNAs by reverse-transcription-polymerase chain reaction (RT-PCR), the cDNA products were separated on 6% sequencing-type gels using a genomixLR sequencer (Genomix Corp.). The dried gels were exposed to Kodak XAR-2 film (Kodak, Rochester, NY) for various times.

Differentially-expressed cDNA fragments were excised and reamplified
15 according to the manufacturer's directions (Genomix Corp.). Because a gel slice excised from the gel contains 1 to 3 cDNA fragments of the same size (Martin *et al.*, *BioTechniques* 24, 1018-26, 1998; Giese *et al.*, Differential Display, Academic Press, 1998), reamplified products were separated by single strand confirmation polymorphism gels as described in (Mathieu-Dande *et al.*, *Nucl. Acids Res.* 24, 1504-07, 1996) and
20 directly sequenced using M13 universal and T7 primers.

Construction and screening of human bone marrow stromal cell cDNA library. RNA was isolated from human bone marrow stromal cells (Poietic Technologies, Inc., Germantown, MD) using a guanidinium thiocyanate/phenol chloroform extraction protocol (Chirgwin *et al.*, *Biochem.* 18, 5294-99, 1979). Poly(A)⁺ RNA was isolated
25 using oligo-dT spin columns (Stratagene, La Jolla, CA). First and second strand synthesis was carried out according to the manufacturer's instructions (Pharmacia, Piscataway, NJ). Double-stranded cDNA was ligated into pBK-CMV phagemid vector (Stratagene, La Jolla, CA). Approximately, 1×10^6 plaques were screened using a 1.2 kb CSP56 cDNA fragment. Plasmid DNA from positive clones was obtained according to
30 the manufacturer's instructions. Correctness of the nucleotide sequence was determined

by double-strand sequencing.

Northern blot analysis and RT-PCR. Northern blots containing poly(A)⁺ RNA prepared from various human normal and tumor tissues were purchased from ClonTech (Palo Alto, CA) and Biochain Institute (San Leandro, CA). All other Northern blots
5 were prepared using 20 to 30 µg total RNA isolated using a guanidinium thiocyanate/phenol chloroform extraction protocol (Chirgwin *et al.*, 1979) from different human breast cancer and normal cell lines. Northern blots were hybridized at 65 °C in Express-hyb (ClonTech).

RT-PCR was performed using the reverse transcriptase RNA PCR kit (Perkin-
10 Elmer, Roche Molecular Systems, Inc., Branchburg, NJ) according to the manufacturer's instructions.

In situ hybridization. *In situ* hybridization was performed on human tissues, frozen immediately after surgical removal and cryosection at 10 µm, following the protocol of Pfaff *et al.*, *Cell* 84, 309-20, 1996. Digoxigenin-UTP-labeled riboprobes
15 were generated using the CSP56-containing plasmid DNA as a template. For generation of the antisense probe, the DNA was linearized with *Eco*RI (approximately 1 kb transcript) or *Nco*I (full-length transcript) and transcribed with T3 polymerase. For the sense control, the DNA was linearized with *Xho*I (full-length transcript) and transcribed with T7 polymerase. Hybridized probes were detected with alkaline phosphatase-
20 coupled anti-digoxigenen antibodies using BM Purple as the substrate (Boehringer Mannheim).

Tumor growth in the mammary fatpad of immunodeficient mice. Scid (severe combined immunodeficient) mice (Jackson Laboratory) were anesthetized, and a small incision was made to expose the mammary fatpad. Approximately 4 x 10⁶ cells were
25 injected into the fatpad of each mouse. Tumor growth was monitored by weekly examination, and growth was determined by caliper measurements. After approximately 4 weeks, primary tumors were removed from anesthetized mice, and the skin incisions were closed with wound clips. Approximately 4 weeks later, mice were killed and inspected for the presence of lung metastases. Primary tumors and lung metastasis were
30 analyzed histologically for the presence of human cells. A chunk of tumor tissue

representing more than 80% cells of human origin was used to isolate total RNA. In the case of MDA-MD-435, large lung metastases representing more than 90% human cells were used. Total RNA was amplified by RT-PCR using specific primers for the CSP56 coding region. The reaction products were dot blotted onto nylon membranes and
5 hybridized with a CSP56-specific probe.

EXAMPLE 1

This example demonstrates identification of a differentially-expressed gene in the aggressive-invasive human breast cancer cell line MDA-MB-435.

10 To identify genes associated with the metastatic phenotype, we compared the gene expression profiles in four human breast cancer cell lines using which display different malignant phenotypes, MDA-MB-453, MCF-7, MDA-MB-231, and MDA-MB-435, ranging from poorly-invasive to most aggressively-invasive (Engel *et al.*, *Cancer Res.* 38, 4327-39, 1978; Shafie and Liotta, *Cancer Lett.* 11, 81-87, 1990; Ozello and
15 Sordat, *Eur. J. Cancer* 16, 553-59, 1980; Price *et al.*, *Cancer Res.* 50, 717-21, 1990). Cell lines were chosen as starting material based on the ability to obtain high amounts of pure RNA. In contrast, human breast cancer biopsies consist of a mixture of cancer and other cell types including macrophages and lymphocytes (Kelly *et al.*, *Br. J. Cancer* 57, 174-77, 1988; Whitford *et al.*, *Br. J. Cancer* 62, 971-75, 1990). The described human
20 breast cancer cell lines have been extensively studied in mouse models allowing one to functionally characterize identified candidate genes in tumor progression.

To ensure that the cell lines retained their original malignant properties after prolonged passage in culture, we examined their potential to grow in scid mice and to form metastasis following injection into the mammary fatpad. Three of the four cell
25 lines formed primary tumors, consistent with previous reports (Engel *et al.*, 1978; Shafie and Liotta, 1990; Ozello and Sordat, 1980; Price *et al.*, 1990). No primary tumor formation was detected with MDA-MB-453. In addition, mice injected with MDA-MB-231 and MDA-MB-435 developed lung metastases, with the highest incidence being detected using MDA-MB-435.

30 Next, we performed a differential display analysis using total RNA isolated from

the breast cancer cell lines and a total of 200 different primer pair combinations. Among several differentially expressed transcripts, a 1.2-kb cDNA fragment was specifically amplified from the MDA-MB-435 RNA sample using the primer pair combination, Ap8 [5'-ACGACTCACTATAGG GC(T)₁₂AA] (SEQ ID NO:20) and Arp1 (5'-

5 ACAATTTTCACACAGGACGACTCCAAG) (SEQ ID NO:21) (Figure 1A, lanes 5 and 6). Weak expression was also detected in MDA-MB-231 (Figure 1A, lanes 1 and 2), whereas no signal was detected in the RNA samples isolated from MCF-7 and MDA-MB-453 (Figure 1A, lanes 3, 4, 7, and 8).

To confirm the expression pattern, the DNA fragment was isolated from the gel, reamplified, radiolabeled, and used as a hybridization probe in a Northern blot analysis of human breast cancer cell lines with different malignant phenotypes and a non-tumorigenic breast cell line (Figure 1B). The radioactive probe hybridized with similar intensity to two transcripts of approximately 2.0-kb and 2.5-kb in size in the MDA-MB-435 RNA sample (lane 9). Weak expression of these transcripts was detected in the poorly invasive human breast cell lines (lanes 2 and 3) or in the non-tumorigenic line Hs578Bst (lane 1). No signal was detected in MDA-MB-453 and MCF-7. These data show a restricted expression pattern of this gene to highly or moderately metastatic human breast cancer cell lines.

EXAMPLE 2

This example demonstrates the nucleotide sequence of CSP56 cDNA.

Comparison of the nucleotide sequence of CSP56 cDNA to public databases showed no significant homologies. To obtain more nucleotide sequence information, we screened a human bone marrow stromal cell cDNA library. One of the positive clones extended the original clone to 1855 nucleotides in length (Figure 2A). This sequence was further extended at the 3'-end with several expressed sequenced tags to 2606 nucleotides in length (Figure 2B). The additional 750 nucleotides are most probably the result of alternative poly-A site selection.

Analysis of the nucleotide sequence revealed a single open reading frame of 518

amino acids, beginning with a start codon for translation at nucleotide position 101 and terminating with a stop codon at nucleotide position 1655. A consensus Kozak sequence (Kozak, *Cell* 44, 283-92, 1986) around the start codon and the analysis of the codon usage (Wisconsin package, UNIX) suggests that this cDNA clone contains the entire coding region.

Translation of the open reading frame predicts a protein with a molecular mass of 56 kD. On the basis of its specific expression in the highly metastatic human breast cancer cell lines, the cDNA-encoded protein was termed CSP56 for cancer-specific protein 56-kd.

EXAMPLE 3

This example demonstrates that CSP56 is a novel aspartyl-type protease.

Comparison of the CSP56 open reading frame with proteins in public databases shows some homology to members of the pepsin family of aspartyl proteases (Figure 3).

A characteristic feature of this protease family is the presence of two active centers which evolved by gene duplication (Davies, *Ann. Rev. Biophys. Biochem.* 19, 189-215, 1990; Neil and Barrett, *Meth. Enz.* 248, 105-80, 1995). The amino acid residues comprising the catalytic domains (Asp-Thr/Ser-Gly) and the flanking residues display the highest conservation in this family and are conserved in CSP56 (Figures 2 and 3).

CSP56, however, shows structural features which are distinct from other aspartyl proteases. Overall similarities of CSP56 to pepsinogen C and A, renin, and cathepsin D and E are only 55, 51, 54, 52, and 51%, respectively, neglecting the CSP56 C-terminal extension. The cysteine residues found following and preceding the catalytic domains in other members are absent in CSP56 (Figure 3). CSP56 also contains a carboxy-terminal extension of approximately 90 amino acid residues which shows no significant homology to known proteins.

CSP56 also contains a hydrophobic motif consisting of 29 amino acid residues in the C-terminal extension which may function as a membrane attachment domain.

(Figures 2C and 3) CSP56 also contains a putative signal sequence.

CSP56 is therefore a novel aspartyl-type protease with a putative transmembrane

domain (amino acids 8-20) and a stretch of approximately 45 amino acids representing a putative propeptide (amino acids 21 to 76).

EXAMPLE 4

5 This example demonstrates the expression pattern of CSP56 throughout human breast cancer development and in metastasis.

To further examine the expression pattern of CSP56, we performed a Northern blot analysis using additional human breast cancer and normal cell lines (Figure 4). Expression of CSP56 was detected in MDA-MB-435, MDA-MB-468, and BR-3 (lanes 1, 10 4, and 9), with the strongest signal in MDA-MB-435. Other cell lines showed weak expression. No signal was detected in the poorly-invasive human breast cancer cell lines MDA-MB-453 and MCF-7 and in a normal breast cell line Hs578Bst. Together, these data are consistent with the increased expression of CSP56 in highly malignant human breast cancer cell lines.

15

EXAMPLE 5

This example demonstrates the expression pattern of CSP56 in normal human tissues.

To determine the tissue distribution of CSP56, polyA⁺ RNA from various human 20 tissues was examined by Northern blot analysis (Figure 7). Two major transcripts were detected that are similar in size to those detected in cancer cell lines and human tissues. Highest expression was detected in pancreas, prostate, and placenta. Weak or no signal was detected in brain and peripheral blood lymphocytes.

25

EXAMPLE 6

This example demonstrates identification of CSP56 transcripts in primary tumors and metastatic lung tissue isolated from immunodeficient mice injected with MDA-MB-435.

The scid mouse model was used to examine CSP56 expression in tumors. This 30 model has been shown to be suitable for evaluating the function of genes implicated in

the tumorigenicity and metastasis of human breast cancer cells (Steeg *et al.*, *Breast Cancer Res. Treat.* 25, 175-87, 1993; Price, *Breast Cancer Res. Treat.* 39, 93-102, 1996).

Different human breast cancer cell lines were injected into the mammary fatpad of immunodeficient mice. Primary tumors and, if applicable, lung metastases were isolated from mice, and total RNA was prepared for Northern blot analysis (Figure 4).

CSP56 transcripts were detected in primary tumor RNA derived from MDA-MB-435, MDA-MB-468 and Alab, but not from MCF-7 (Figure 4). CSP56 gene expression was also detected in lung metastasis of mice injected with MDA-MB-435 (lane 1). Failure to detect CSP56 transcripts in primary tumors of mice injected with ZR-75-1, MDA-MB-361, and MDA-MB-231 could be explained with the small amount of human cancer tissues in these tumors as judged by the weak human β -actin signal when compared to other primary tumor RNA samples.

Together these data exclude *in vitro* culture conditions as a cause for CSP56 up-regulation and establishes this gene as a novel tumor maker.

EXAMPLE 7

This example demonstrates detection of CSP56 gene expression detected in patient samples.

CSP56 expression was examined in RNA samples isolated from patient tumor biopsies. A Northern blot containing total RNA from breast tumor tissue and normal breast tissue from the same patient was hybridized with a CSP56-specific probe (Fig. 5A). CSP56 transcripts were detected in the tumor sample whereas no signal was detected in the normal breast RNA (lanes 1 and 2). Similarly, expression of CSP56 transcripts were up-regulated in two other breast cancer RNA samples when compared to a normal breast RNA control (Fig. 5B). Increased expression of CSP56 was also detected in human colon cancer tissue when compared to normal colon tissue of the same patient.

To identify the cell types that express CSP56 transcripts *in vivo*, we performed an *in situ* hybridization analysis on tissue samples obtained from one breast cancer patient (Figure 6A-6F). A weak CSP56 signal was detected in the cells of the ducts of normal

breast tissue (Figure 6B). In the primary tumor, CSP56 was highly expressed in the tumor cells but not in the surrounding lymphocytes (Figure 6E). No signal was detected using the sense probe (Figures 6C and 6F).

5 We also analyzed tissue samples obtained from two colon cancer patients (Figures 6G-6M) for CSP56 expression. No signal was detected in normal colon tissue (Figure 6H), whereas CSP56 transcripts were abundant in the tumor cells of both the primary colon tumor and the liver metastasis, and no expression was detected in the surrounding stroma (Figures 6K and 6M).

10 These data demonstrate that CSP56 is over-expressed in tumor cells of human cancer patients and may play a role in the development and progression of different types of tumors.

Table 1.

TRANSCRIPT NUMBER	SEQ ID NO: and Figure No.	non- metastatic breast	breast cancer metastatic to bone	breast cancer metastatic to lung	low metastatic from colon	high metastatic from colon
122	1	-	-	+		
156	2	+	-	-		
166	3	+	-	-		
172	4	-	-	+		
245	5	+	+	-		
280	6	+	-	-		
288	7	+	-	-		
337	8	+	-	-		
344	9	+	-	-		
355	10	+	-	-		
42	11	-	-	+		
59	12	+	-	-		
87	13	+	-	-		
310	14	+	+	-		
349	15	+	-	-		
362c	16				-	+
305c	17				+	-

+ indicates that the transcript is detectable in Northern blots.

- indicates that the transcript is not detectable in Northern blots.

Some transcripts are detectable upon RT-PCR even when not detectable in Northern blots.

CLAIMS

1. An isolated and purified protein having an amino acid sequence which is at least 85% identical to an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18, wherein percent identity is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.
2. The isolated and purified protein of claim 1 which is at least 85% identical to the amino acid sequence shown in SEQ ID NO:19.
3. The isolated and purified protein of claim 1 which comprises an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.
4. The isolated and purified protein of claim 2 which comprises the amino acid sequence shown in SEQ ID NO:19.
5. An isolated and purified polypeptide which consists of at least 8 contiguous amino acids of a protein having an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.
6. The isolated and purified polypeptide of claim 5 which consists of at least 8 contiguous amino acids of SEQ ID NO:19.
7. The isolated polypeptide of claim 6 which is selected from the group consisting of at least amino acids 461-489 of SEQ ID NO:19, at least amino acids 106-115 of SEQ ID NO:19, at least amino acids 297-306 of SEQ ID NO:19, and at least amino acids 8-20 of SEQ ID NO:19.
8. A fusion protein which comprises a first protein segment and a second protein segment fused to each other by means of a peptide bond, wherein the first protein segment consists of at least 8 contiguous amino acids selected from an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

9. The fusion protein of claim 8 wherein the first protein segment consists of at least 8 contiguous amino acids selected from the amino acid sequence shown in SEQ ID NO:19.
10. A preparation of antibodies which specifically bind to a protein with an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.
11. A cDNA molecule which encodes an isolated and purified protein having an amino acid sequence which is at least 85% identical to an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1-18, wherein percent identity is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.
12. The cDNA molecule of claim 11 which encodes a protein having an amino acid sequence which is at least 85% identical to SEQ ID NO:19.
13. A cDNA molecule which encodes at least 8 contiguous amino acids of a protein encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.
14. The cDNA molecule of claim 13 which encodes SEQ ID NO:19.
15. The cDNA molecule of claim 14 which comprises SEQ ID NO:18.
16. A cDNA molecule comprising at least 12 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.
17. A cDNA molecule which is at least 85% identical to a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18, wherein percent identity is determined using a Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 1.
18. The cDNA molecule of claim 17 which is at least 85% identical to the nucleotide sequence shown in SEQ ID NO:18.
19. An isolated and purified subgenomic polynucleotide comprising a nucleotide segment which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18 after washing with 0.2 X SSC at 65 °C.

20. The isolated and purified subgenomic polynucleotide of claim 19 wherein the nucleotide segment hybridizes to a nucleotide sequence as shown in SEQ ID NO:18.

21. A construct comprising:

a promoter; and

a polynucleotide segment encoding at least 8 contiguous amino acids of a protein encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18, wherein the polynucleotide segment is located downstream from the promoter, wherein transcription of the polynucleotide segment initiates at the promoter.

22. The construct of claim 21 wherein the protein comprises the amino acid sequence of SEQ ID NO:19.

23. A host cell comprising a construct which comprises:

a promoter and:

a polynucleotide segment encoding at least 8 contiguous amino acids of a protein encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

24. The host cell of claim 23 wherein the protein has the amino acid sequence shown in SEQ ID NO:19.

25. A recombinant host cell comprising a new transcription initiation unit, wherein the new transcription initiation unit comprises in 5' to 3' order:

(a) an exogenous regulatory sequence;

(b) an exogenous exon; and

(c) a splice donor site,

wherein the new transcription initiation unit is located upstream of a coding sequence of a gene, wherein the coding sequence comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18, wherein the exogenous regulatory sequence controls transcription of the coding sequence of the gene.

26. The recombinant host cell of claim 25 wherein the gene has the coding sequence shown in SEQ ID NO:18.

27. A polynucleotide probe comprising (a) at least 12 contiguous nucleotides

selected from the group consisting of SEQ ID NOS:1-18 and (b) a detectable label.

28. The polynucleotide probe of claim 27 wherein the at least 12 contiguous nucleotides are selected from SEQ ID NO:18.

29. A method for identifying a metastatic tissue or metastatic potential of a tissue, comprising the step of:

measuring in a tissue sample an expression product of a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-4, 6-13, and 15-18, wherein a tissue sample which expresses a product of a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1, 4, 11, 16, 17, and 18 or which does not express a product of a gene comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, and 15 is identified as metastatic or as having metastatic potential.

30. The method of claim 29 wherein the tissue sample is selected from the group consisting of breast and colon tissue.

31. The method of claim 29 wherein the expression product is protein.

32. The method of claim 29 wherein the expression product is mRNA.

33. The method of claim 29 wherein the gene comprises the nucleotide sequence shown in SEQ ID NO:18.

34. A method of screening test compounds for the ability to suppress the metastatic potential of a tumor, comprising the steps of:

contacting a biological sample with a test compound; and

measuring in the biological sample the synthesis of a protein having an amino acid sequence encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-4, 6-13, and 15-18, wherein a test compound which decreases synthesis of a protein encoded by a polynucleotide comprising SEQ ID NOS:1, 4, 11, 16, 17, or 18 or which increases synthesis of a protein encoded by a polynucleotide comprising SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, or 15 is identified as a potential agent for suppressing the metastatic potential of a tumor.

35. A method of predicting propensity for high-grade or low-grade metastatic spread of a colon tumor, comprising the steps of:

measuring in a colon tumor sample an expression product of a gene having a sequence selected from the group consisting of SEQ ID NOS:16 and 17, wherein a colon tumor sample which expresses the product of SEQ ID NO:16 is categorized as having a high propensity to metastasize and a colon tumor sample which expresses the product of SEQ ID NO:17 is categorized as having a low propensity to metastasize.

36. A set of primers for amplifying at least a portion of a gene having a coding sequence selected from the group consisting of the nucleotide sequences shown in SEQ ID NOS:1-18.

37. The set of claim 36 wherein the gene has the coding sequence shown in SEQ ID NO:18.

38. The set of claim 37 wherein the primers are the nucleotide sequences shown in SEQ ID NOS:20 and 21.

39. A polynucleotide array comprising at least one single-stranded polynucleotide which comprises at least 12 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-18.

40. The polynucleotide array of claim 40 wherein the nucleotide sequence is selected from the group consisting of SEQ ID NOS:1, 4, 11, 16, 17, and 18.

41. The polynucleotide array of claim 40 wherein the nucleotide sequence is selected from the group consisting of SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, and 15.

42. A method of identifying a metastatic tissue or metastatic potential of a tissue, comprising the steps of:

contacting a tissue sample comprising single-stranded polynucleotide molecules with a polynucleotide array comprising at least one single-stranded polynucleotide probe, wherein the at least one single-stranded polynucleotide probe comprises at least 12 contiguous nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOS:1-4, 6-13, and 15-18, wherein the tissue sample is suspected of being metastatic or of having metastatic potential;

detecting double-stranded polynucleotides bound to the polynucleotide array, wherein detection of a double-stranded polynucleotide comprising contiguous

nucleotides selected from the group consisting of SEQ ID NOS:1-4, 11, 16, 17, and 18 or lack of detection of a double-stranded polynucleotide comprising contiguous nucleotides selected from the group consisting of SEQ ID NOS:2, 3, 6, 7, 8, 9, 10, 12, 13, and 15 identifies the tissue sample as metastatic or of having metastatic potential.

43. The method of claim 42 wherein the tissue sample is a breast or colon sample.

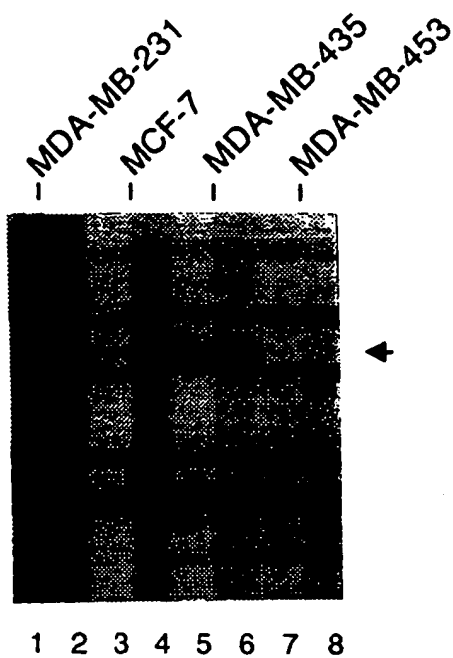


FIG. 1A

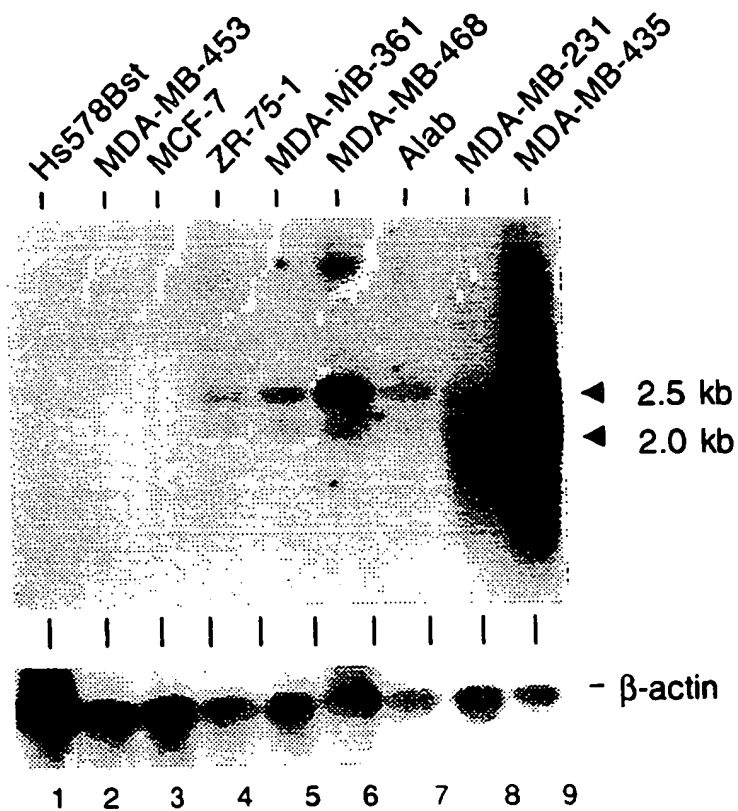


FIG. 1B

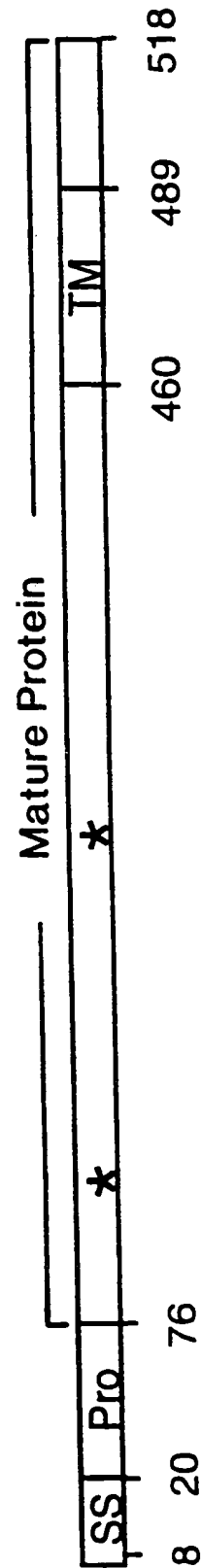
FIG. 2A-2

TATTAAGGAAGAGTGGTACTACCAGATAGAAATTCGAAATTTGGAGGCCAAAGCCCTTAATCTGGACTGCAGAGAGTATAACGACAGACAAGGCC 1000
I K E E W Y Y Q I E I L K L E I G G Q S L N L D C R E Y N A D K A 297
ATCGTGGACAGTGGCACCACGCTGCTGGCCCTGCCCCAGAGGTTGATGCGGTGGTGAAGCTGTGGCCCGCCGCAATCTCTGATTCAGAAATCTCTG 1100
I V D S G T T L L R L P Q K V F D A V V E A V A R A S L I P E F S 330
ATGGTTTCTGGACTGGTCCCAGCTGGGTGGACGAATTCGGAAACACACCTTGGTCTTACTTCCCTAAATCTCCATCTACCTGAGAGATGAGAATC 1200
D G F W T G S Q L A C W T N S E T P W S Y F P K I S I Y L R D E N S 363
CAGCAGGTCAATTCGGTATCACAATCCTGCCTCAGCTTTACATTCAGCCCATGATGGGGCGCGCTGAATTAATGATGTTACCGAATTCGGCAATTCGCCA 1300
S R S F R I T I L P Q L Y I Q P M M G A G L N Y E C Y R F G I S P 396
TCCACAAATGCGCTGGTGATCGGTGCCACGGTGATGGAGGGCTTCTACGTCATCTTCGACAGAGCCCAAGAGGGTGGCTTCGCCAGCGGCCCTGTG 1400
S T N A L V I G A T V M E G F Y V I F D R A Q K R V G F A A S P C 429
CAGAAATTCAGGTGCTGAGTGTGAAATTTCCGGGCCCTTCTCAACAGAGGATGTAGCCAGCAACTGTGTCCCCGCTCAGTCTTTGAGCGAGCCCAT 1500
A E I A G A A V S E I S G P F S T E D V A S N C V P A Q S L S E P I 462
TTTGTGATTTGTCCCTATGCGCTCATGAGCGTCTGTGGAGCCATCCTCTGTCTTAATCGTCTGCTGCTGCTGCCGTTCGGGTGTCAGCGTCCGCC 1600
L W I V S Y A L M S V C G A I L L V L I V L L L P F R C Q R R P 495
CGTGACCCCTGAGGTGCTCAATGATGAGTCTCTCTGGTCAGACATCGCTGGAATAGCCAGGCCCTGACCTCAAGCAACCATGAACCTCAGCTATTAA 1700
R D P E V V N D E S S L V R H R W K * 513
GAAATCACATTTCCAGGCGCAGCGGGATCGATGGTGGCGCTTTCTCTGTGCCACCCGCTTCAATCTCTGTCTCTCTCCAGATGCCCTCTCTAGA 1800
TTCACTGTCTTTTGTGATTTTCAAGCTTTTCAATCTCTCCCTACTTCCAAG(A)n 1855

FIG. 2B

1955 AAAAAAAAAA CTTCA TTTCTAA ACCAAACAGAGTGGATTGGCTGCAGGCTCTATGGGGTTTCGTTATGCCAAAAGTGTCTACATGTGCCACCAACATAAAA
2055 CAAAACCAAGCCTTGGCTCGTTCTCTCTCTCTCAATCTCTTGGAAAAATAAGTACATATAGTTGATTAACCCCTCTTAGCTTACAGGAAGCTTTTGTGTAT
2155 TAAATGCCCTTTGAGGTTATTTTCCGCCAGACCTCAACCTGGGTCAAAGTGGTACAGGAAGGCTTCAGTATGTCAGGAGGAATCAGCCTGGGGCCTGG
2255 GGATGTAACCAAGCTGTACCCCTTGAGACCTGGAAACAGAGGCCACAGGCCCTCTTTTGTGGTTTCTCTGTGCTCTGAATGGGAGCCAGAAATTCACTAGGAG
2355 GTCATCAACCGATGGTCTCACAAGCCTCTCTCTGAAGATGGAAGGCCTTTTCGCCCTGTGAGGTAGAGGGGAAGGAATCTCTCTTTTGTACCCCAATACT
2455 TATGTTGTATTTGTTGGTGCAGAAAGTAAACACTACCTCTTTTGAGACTTTGCCCAGGGTCTGTGCCGTGGATGGGGTGCAGGCAGCCTTGACCAACGGC
2555 TGTTCCTCTACCCCAAAAGAAATTAATCTCCCAACAGCCAAAGACCCCAACAGGTGCTGAAC TGTGCATCAACACGGAAGAGTTCTATCCCCCAAGCTGGCCAC
TATCACATATGCTTACTCTTTGCTTAAAAATTAATAAATCATGTTTGTGATGAG (A) n 2606

FIG. 2C



SUBSTITUTE SHEET (rule 26)

FIG. 3B

CatE	E G L T V	V G Q F G E S V T	E P G Q T F V D A P	F D G I L G L G Y P	S L A . .	193
PepA	G C I S D	T N Q E L S E T	E P G S F F Y A P	F D G I L G L A Y P	S I S . .	191
PepC	Q S I Q V	P N Q E L S E N	E P G T N F Y A Q	F D G I M G L A Y P	A L S . .	188
CatD	S A S A L G G	G V K V	E R Q V G E A T K	Q P G I T F I A A K	F D G I L G L A Y P	R I S . .	207
Renlin	G G I T V	T . Q M F G E V T E	M P A L P F M L E E	F D G V V G G E I	E Q A . .	202
CSP56	S F L V N I A T I F	E S E N F	E S E N F	M N G I L G L A Y A	T L A K P	207

	CatE	PepA	PepC	CatD	RenIn	CSP58																	
VGGVT	PVF	FDN	MMMA	Q	N	LVDL	PM	F	SV	Y	M	SS	NP	EG	GA	GS	EL	I	F	233			
SSGAT	PVF	FDN	IWN	Q	G	LVSQ	DL	F	SV	Y	L	S	A	DD	QS	GS	V	I	F	229			
VDEAT	TAM	QGM	VQ	E	G	ALTS	PV	F	SV	Y	L	S	N	Q	SS	GS	A	V	V	F	227		
VNNVL	PVF	FDN	LMQ	Q	K	LVDQ	NI	F	SF	Y	L	S	R	DP	Q	P	GS	E	L	M	L	247	
IGRVT	PIF	FDN	IIS	Q	G	VLKE	DV	F	SF	Y	Y	N	R	DS	S	Q	SL	G	G	I	V	L	244
SSSLE	TF	FDS	LVT	Q	A	NIP	NV	F	SM	Q	M	C	G	AG	LP	V	AG	SG	SL	V	L	251	

Cate	G G Y D S H P F S G	S L N W V P V T K Q	A Y W Q I A L D N I	V M . F C	E G C Q
PepA	G G I D S S Y Y T G G	S L N W V P V T E	G Y W Q I T V D S I	F C	Q
PepC	G G V D S S L Y T G G	Q I Y W A P V T Q E	L Y W Q I G I E E F	. A C	Q
CalD	G G T D S K Y Y K G	S L S Y L N V T R K	A Y W Q I H L D Q V	A S G W C	Q
RenIn	G G S D P Q H Y E G	N F H Y I N L I K T	G V W Q I Q M K G V	. L L C	E
CSP56	G G I E P S L Y K G	D I W Y T P I K E E	W Y W Q I E I L K L	I	L

[illegible]

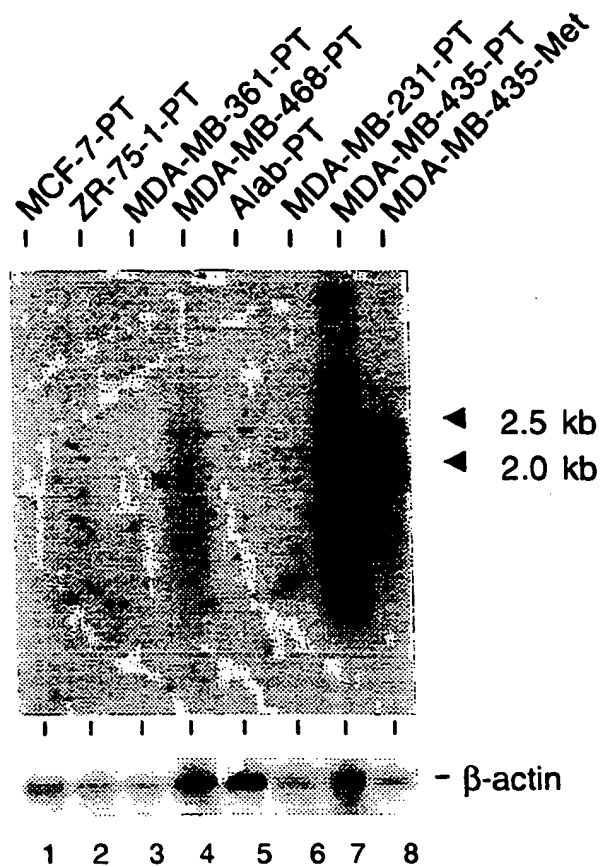


FIG. 4

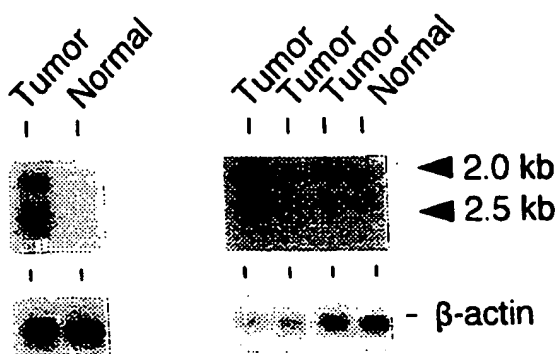
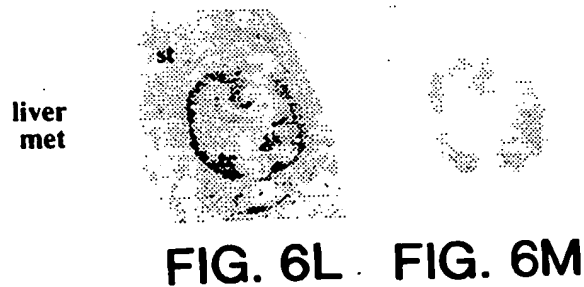
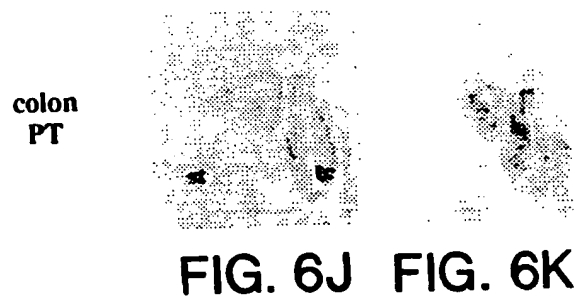
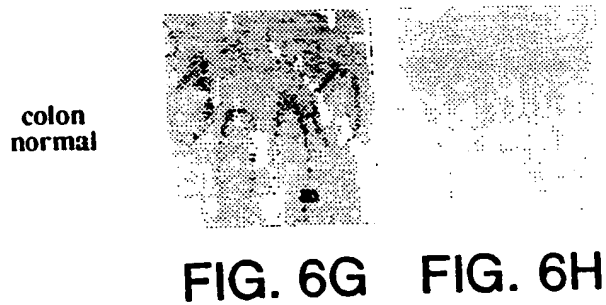
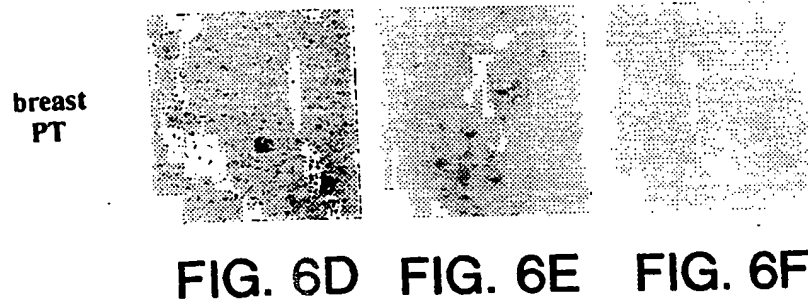
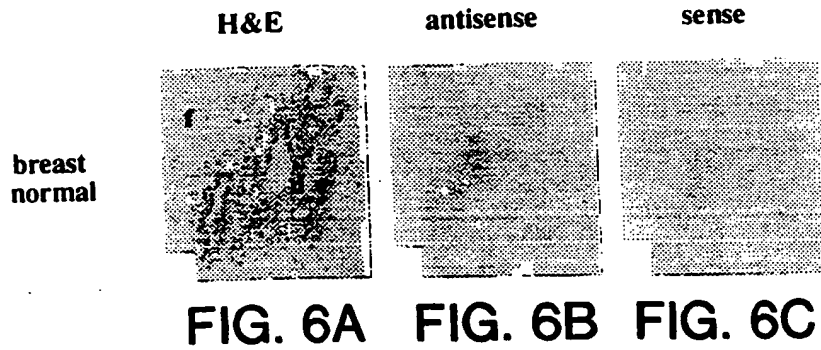


FIG. 5A

FIG. 5B



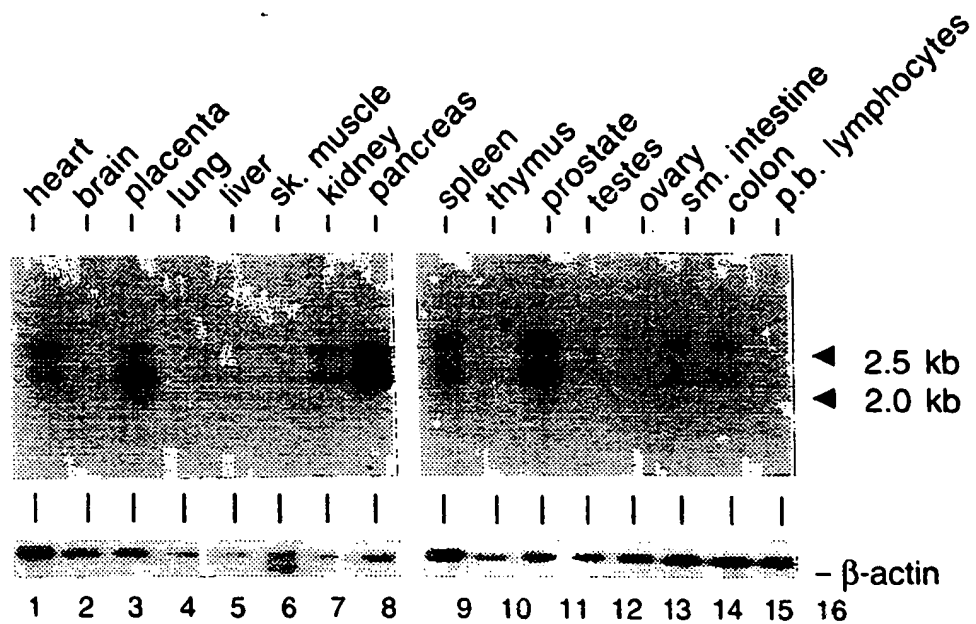


FIG. 7

SEQUENCE LISTING

<110> Chiron Corporation

<120> Metastatic Breast and Colon Cancer
Regulated Genes

<130> 1451.100

<140> PCT/US98/27608

<141> 1998-12-24

<160> 21

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 2429

<212> DNA

<213> human

<400> 1

acaagttgca	cttaagaagc	tatgctaaga	aaacaaacac	acagaagcct	acatcattac	60
atgtatagaa	tgttcaagaa	ctgatgaaac	cagtcctgtg	tcacaaaagc	cagaaagtgg	120
ttgcttcttg	ggaccagaag	ggaaaggggc	ataaaggaaac	cttttgaggt	gaatagaagt	180
ttctgcatct	tggtttggca	cacatgccaa	aactcaccag	ctacagattc	tcgttgacac	240
tggaagcagt	aactttgccg	tgcaaggaaac	cccgcaactcc	tacatagaca	cgtactttga	300
cacagagagg	tctagcacat	accgctccaa	gggctttgac	gtcacagtga	agtacacaca	360
aggaagctgg	acgggcttcg	ttggggaaga	cctcgtcacc	atcccaaaag	gcttcaatac	420
ttcttttctt	gtcaacattg	ccactatttt	tgaatcagag	aatttctttt	tgcttgggat	480
taaatggaat	ggaatacttg	gcctagctta	tgccacactt	gccaagccat	caagttctct	540
ggagaccttc	ttcgactccc	tggtgacaca	agcaaacatc	cccaacgttt	tctccatgca	600
gatgtgtgga	gcccgtttgc	ccgttgctgg	atctgggacc	aacggaggta	gtcttgtctt	660
gggttggaatt	gaaccaagtt	tgtataaagg	agacatctgg	tataccccta	ttaagggaaga	720
gtggtactac	cagatagaaa	ttctgaaatt	ggaaattgga	ggccaaagcc	ttaattctgga	780
ctgcagagag	tataacgcag	acaaggccat	cgtggacagt	ggcaccacgc	tgctgcgcct	840
gccccagaag	gtggttgatg	cggtgggtgga	agctgtggcc	cgcgcatctc	tgattccaga	900
attctctgat	ggttttctgga	ctgggtccca	gctggcgtgc	tggaacgaatt	cggaaacacc	960
ttggtcttac	ttccctaaaa	tctccatcta	cctgagagat	gagaactcca	gcaggtcatt	1020
ccgtatcaca	atcctgcctc	agcttttacat	tcagcccata	atggggggccg	gcctgaatta	1080
tgaatgttac	cgattcggca	tttccccatc	cacaaatgcg	ctgggtgatcg	gtgccacggg	1140
gatggagggc	ttctacgtca	tcttcgacag	agcccagaag	agggtgggct	tcgcagcgag	1200
cccctgtgca	gaaattgcag	gtgctgcagt	gtctgaaatt	tccgggcctt	tctcaacaga	1260
ggatgtagcc	agcaactgtg	tcccgcctca	gtctttgagc	gagcccattt	tgtggattgt	1320
gtcctatgcg	ctcatgagcg	tctgtggagc	catcctcctt	gtcttaatcg	tcctgctgct	1380
gctgccgttc	cggtgtcagc	gtcgcccccg	tgaccctgag	gtcgtcaatg	atgagtcctc	1440
tctggtcaga	catcgctgga	aatgaatagc	caggcctgac	ctcaagcaac	catgaactca	1500
gctattaaga	aaatcacatt	tccagggcag	cagccgggat	cgatggtggc	gctttctcct	1560
gtgcccaccc	gtcttcaatc	tctgttctgc	tcccagatgc	cttctagatt	cactgtcttt	1620
tgattcttga	ttttcaagct	ttcaaatcct	ccctacttcc	aagaaaaata	attaaaaaaa	1680
aaacttcatt	ctaaaccaaa	acagagtggg	ttgggtctca	ggctctatgg	ggttcgttat	1740
gccaaagtgt	ctacatgtgc	caccaacata	aaacaaacac	agccttgggc	tcgttctctt	1800
ctctcttcaa	tctctggaaa	aataagtaca	tatagctgat	ttccctctct	agcttacagg	1860
aagctttttg	tattaattgc	ctttgaggtt	attttccgct	agacctcaac	ctgggtcaaa	1920
gtggtacagg	aaggcttgca	gtatgatggc	aggagaatca	gcctggggcc	tggggatgta	1980
accaagctgt	acccttgaga	cctggaacca	gagccacagg	ccctctctgt	gggtttctct	2040

```

gtgctctgaa tgggagccag aattcactag gaggtcatca accgatggc ctcacaagcc 2100
tcttctgaag atggaaggcc ttttgcccg tgaggtagag gggaaggaaa tctcctcttt 2160
tgtaccaaat acttatgttg tattgttggt gcgaaagtaa aaacactacc tcttttgaga 2220
ctttgccag ggtcctgtgc ctggatgggg gtgcaggcag ccttgaccac ggctgttccc 2280
ctcaccctaaa agaattatca tcccaacagc caagaccctaa caggtgtctga actgtgcatc 2340
aaccagggaag agttctatcc ccaagctggc cactatcaca targcttact cttgcttaaa 2400
attaataaat catgttttga tgagaraaa 2429

```

<210> 2

<211> 486

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1)...(486)

<223> n = A,T,C or G

<400> 2

```

tgtggwtggt ctcctagcat gttaatagat ataactcaca taaaaaatta ttgaggtctt 60
caataatttt tttttttraa caggggaactc tctctgttgc ccaggctgga ttgcattggc 120
acaatcacgg ctcaactggag gcctcaattg cctgggctca attaatccc tcatcttacc 180
ctcccaatta cctgggacca caaacttttg ccaccaggct gggttattat ttttaatac 240
aaggctctcg tattttggcc aaactgggtc caaatycctg ggctcaacca atccyctccc 300
catttcctcc caaatttctg ggattacagg cttaagctac cacacctggc cagccctcaa 360
taatttttaa aattaaaaaa attctcctaa acccaaaaat ttaaggacc tktaaggtag 420
aaaaaaacta tthtyaaaaa aattttctac tcccyctmmmm aaaaaaaaaa ccccnntttt 480
tttttt 486

```

<210> 3

<211> 397

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1)...(397)

<223> n = A,T,C or G

<400> 3

```

tggtatctga canaataasr atgcamccat tkttganggg gtawtattta tctcagggat 60
ttactgtaaa tatgtataca cacatacaaa aaccaggca ttgttaagag aaaataatgg 120
cccaragggt gaaattatca gacagaacct ttaaaaaataa ttatgattaa tgtgttaaaa 180
ttctagtggg aaagataaat aacatgctca ggaaatttta gcagagagat agaaactatg 240
tggaagctc aaatgaaaat gctaggaaat gaaaagcagt attggagggt aaagattcct 300
ttggcaattt atcaacanac tggagatggc anaggcataa tcagtantat tgaaggcaga 360
ttactatnta ttatncaanc aaaaaaaaaa accccct 397

```

<210> 4

<211> 376

<212> DNA

<213> human

<400> 4

```

gtttctactt gaaagtactg atcaaagtga gcattaccag gtatggacaa cttgatatta 60
tgggtatat tctcatcta ggactgccat aacaaaacac cacagactag gagccttaaa 120
caacagaaac ttattttctc acggttctga aggggtggaa gtccaagatc gtgggtgtcaa 180
caggcttggg ttctcccgag gctcaccctc ttggcttgca gacaacagcc tttttatagc 240

```

atcctcctac	ggcctttcct	ctgcgcgtga	gcactcccag	tgtctgtctc	tctcacctgt	300
tgtaagaaca	ccaatcttat	tggtatgctat	aggcctccac	ccttatgacg	tcattaaact	360
ttaaatgccg	gtttaa					376

<210> 5
 <211> 380
 <212> DNA
 <213> human

<400> 5						
tttygtttta	gatagcaagg	cactagaact	ggaaaagaca	cagaaaaaca	aagaatccaa	60
ccctttcatc	ttacaggtga	aacaaactgt	gatgatgcac	atgtatgtgt	tttgtaagct	120
gtgagcaccg	taacaaaatg	taaatttgcc	attatttagga	agtgtctggtg	gcagtgaaga	180
agcaccacag	ccacttgact	cccagtcctg	tgccctgtct	acaccagaca	acacaggagc	240
tggttcagat	tcccctcagc	tgcttaacaa	agttcctcga	acagaaaagt	gcttacaaag	300
ctgccttctc	ggatactgga	aaggctcagt	tttctgaact	gcactgattt	tattgcagtt	360
gaaaaaaaaa	aaacccttwt					380

<210> 6
 <211> 2730
 <212> DNA
 <213> human

<400> 6						
cttgattacg	ccaagctcga	aattaaccct	cactaaaggg	aacaaaagct	ggagctcgcg	60
cgccctgcag	tcgacactag	tggtatccaa	gaattcggca	cgagacgtga	ggggccccc	120
cgtggaagcc	ggctgtctga	atccccacat	cgtcctcaac	attgacctgg	ccccaccat	180
cctggacatt	gcaggcctgg	acataacctgc	ggatatggac	gggaaatcca	tcctcaagct	240
gctggacacg	gagcgccggg	tgaatcggtt	tcacttgaaa	aagaagatga	gggtctggcg	300
ggactccttc	ttggtggaga	gaggcaagct	gctacacaag	agagacaatg	acaaggtgga	360
cgcccaggag	gagaactttc	gcccaggtac	cagcgtgtga	aggacctgtg	tcagcgtgct	420
gagtaccaga	cggcgtgtga	gcagctggga	cagaagtggc	agtgtgtgga	ggacgccacg	480
gggaagctga	agctgcataa	gtgcaagggc	cccatgcggc	tgggcgccag	cagagccctc	540
tccaacctcg	tgcccagta	atacgggcag	ggcagcgagg	cctgcacctg	tgacagcggg	600
gagtaacaag	tcagcctggc	cgagcgccgg	aaaaaactct	tcaagaagaa	gtacaaggcc	660
agctatgtcc	gcagtcgctc	catccgctca	gtggccatcg	aggtggacgg	cagggtgtac	720
cacgtaggcc	tggtgtgatc	cgcccagccc	cgaaacctca	ccaagcggca	ctggccaggg	780
gcccctgagg	accaagatga	caaggatggt	ggggacttca	gtggcactgg	aggccttccc	840
gactactcag	ccgccaaccc	cattaaagtg	acacatcgca	ggtgctacat	cctagagaac	900
gacacagtcc	agtgtgacct	ggacctgtac	aagtcacctg	aggcctggaa	agaccacaag	960
ctgcacatcg	accacgagat	tgaaaccttg	cagaacaaaa	ttaagaacct	gagggaagtc	1020
cgaggtcacc	tgaagaaaaa	gcggccagaa	gaatgtgact	gtcacaaaaat	cagctaccac	1080
accagcaca	aaggccgcct	caagcacaga	ggctccagtc	tgcattcctt	caggaagggc	1140
ctgcaagaga	aggacaaggt	gtggctgttg	cgggagcaga	agcgcaagaa	gaaactccgc	1200
aagctgctca	agcgctgca	gaacaacgac	acgtgcagca	tgccaggcct	cacgtgcttc	1260
accacgcaca	accagcactg	gcagacggcg	cctttctgga	cactggggcc	tttctgtgcc	1320
tgcaccagcg	ccaacaataa	cacgtactgg	tgcattgagga	ccatcaatga	gactcacaat	1380
ttcctcttct	tggaatttgc	aactggcttc	ctagagtact	ttgatctcaa	cacagacccc	1440
taccagctga	tgaatgcagt	gaacacactg	gacaggggatg	tcctcaacca	gctacacgta	1500
cagctcatgg	agctgaggag	ctgcaagggt	tacaagcagt	gtaacccccg	gactcgaaac	1560
atggacctgg	gacttaaaga	tggaaggaagc	tatgagcaat	acaggcagtt	tcagcgtcga	1620
aaagtggccag	aaatgaagag	accttcttcc	aaatcactgg	gacaactgtg	ggaaggctgg	1680
gaagggttaag	aaacaacaga	ggtggacctc	caaaaacata	gaggcatcac	ctgactgcac	1740
aggcaatgaa	aaacctatgt	ggtgatttcc	agcagacctg	tggtattggc	caggaggcct	1800
gagaaagcaa	gcacgcactc	tcagtcacaa	tgagagattc	tggaaggataa	ccagcaggag	1860
cagagataaac	ttcagggaagt	ccatttttgc	ccctgctttt	gctttggatt	atacctcacc	1920
agctgcacaa	aatgcatttt	ttcgtatcaa	aaagtcacca	ctaaccctcc	cccagaagct	1980
cacaaaaggaa	aacggagaga	gcgagcgaga	gagatttctt	tggaatttcc	tcccaagggc	2040

```

gaaagtcatt ggaattttta atcatagggg aaaagcagtc ctgttctaaa tccctcttatt 2100
cttttggttt gtcacaaaga aggaactaag aagcaggaca gaggcaacgt ggagaggctg 2160
aaaacagtgc agagacgttt gacaatgagt cagtagcaca aaagagatga catttaccta 2220
gcactataaa ccctggttgc ctctgaagaa actgccttca ttgtatatat gtgactatct 2280
acatgtaatc aacatgggaa ctttttagggg aacctataaa gaaatcccaa ttttcaggag 2340
tgggtggtgc aataaacgct ctgtggccag tgtaaaagaa aatccctcgc agttgtggac 2400
atctctgttc ctgtccagat accatttctc ctagtatttc tttgttatgt cccagaactg 2460
atgttttttt ttttaaggta tgaaaagaaa tgaagtgtat gtatgtccca agttttgatg 2520
aaactgtatt agtaaaaaaa atttttagtg ttaagtattg tcatacagtg ttcaaaaccc 2580
cagccaatga ccagcagttg gtatgaagaa cctttgacat tttgtaaaag gccatttctt 2640
ggggaaaaaa aaaaaaaaaa aaaaaaaaaa aactcgagag tacttctaga gcggccgcgg 2700
gccccatgat tttccacccg ggtgggggat 2730

```

<210> 7

<211> 218

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1) ... (218)

<223> n = A,T,C or G

<400> 7

```

ttntccatga ctcggggtcn cnnatggcat caaacaggan gnngnggctt catngtaaan 60
naccgtnatn tctnctncgg tccggtgtcc atnttgccn tcngacatcc tggtangacg 120
ccgagacaat ataaatgtac aatggatacc cgatgcaaac aatgtattgt ggtaactag 180
gtgtnatccc ncccattgtg ntantaaggg cngntgtc 218

```

<210> 8

<211> 426

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1) ... (426)

<223> n = A,T,C or G

<400> 8

```

gtyyatgatc acatctgacg ctattcctat ccccttcctc cccgggacct tttccccttc 60
ctccctggga ccttttcccc ttctgttta anaagccagg gctgcctgga ggaagctttg 120
tcagatctag tggaatgtga cctccctgga atatgtgccc aggggtttgt ctaagcagtt 180
tcaggctatg gcctttactc catctggtcc ccateccctc tatctctctc atgtgtggct 240
gcacctggac gcttggacca tagctgtcac agccccctgg ggaggaaccc actccttggc 300
catgtcagcc tgtgcaatgc aaggctcttg tttgatctgt gtgctgacan aaagcccage 360
ttccttaaga acttttcatg tggaacactt tggttttgag aagaaaataa atcanaaacc 420
attaa 426

```

<210> 9

<211> 480

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1) ... (480)

<223> n = A,T,C or G

<400> 9

ctctaccctt	tcctgatcca	tgatcggggt	cgcctttgga	gcanaagga	ggcnatggcc	60
acatgganaa	cnaggtgatc	tgccccctgg	tcctgggtgc	cangctggcc	ctcggcnccc	120
tgggcnaagc	nccnactnag	acntntanna	nncccccccg	gantanaent	aatgntagnt	180
ctnctnntnt	cccnccctcc	ccntnttctn	nttaaggctg	cnntttccnc	tacacenncc	240
ntgnggtcnc	nngnnncttc	cntcctagtg	tnctctantt	ccttcccnat	gacgattgtc	300
aattacagac	acccccntca	cgcangtggtg	agggacgaac	nccggtgcct	ccgtcactct	360
gggggcnatt	nncataccnt	ggaatttaac	ccntttctna	ctgttcttnt	ttgaatnnat	420
tgtntgtnc	agnttttgtt	caatattgat	aagctacgta	tttanaaaat	atcatgctgt	480

<210> 10

<211> 402

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1)...(402)

<223> n = A,T,C or G

<400> 10

tgcatacagg	gaattaacaa	atatatgaag	tgtttcatga	tcctccatca	gtttttaaat	60
atgtctaatt	aactcattta	cctagaaaaa	tataattgtc	gatgagtttt	taatgtgagg	120
agaasagctc	ggctctcggc	atctgtccac	gtgcaggagc	cacttgggag	tgatcatttc	180
aagcaggggt	cttgagagagc	caggctgagg	ccaggtcatt	ttgggctggt	tgcaatccta	240
actgggtcag	ggcgaggcag	gccagtgaag	ggattaaaac	tcttcaccct	ctctaggccc	300
gtgttctgcc	tcycwttag	cactcatctg	tmrcttggtt	tagtccctgg	tcanccaagg	360
ggggaattcc	tggcccctgt	caaaattctc	aggaggctcc	aa		402

<210> 11

<211> 575

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1)...(575)

<223> n = A,T,C or G

<400> 11

ttgcacagga	gcatggnaga	atgatgaact	tccgtcagcg	gatgggatgg	attggagtgg	60
gattgtatct	gttagccagt	gcagcagcat	tttactatgt	ttttgaaatc	agtgagactt	120
acaacaggct	ggccttgga	cacattcaac	agcaccctga	ggagcccctt	gaaggaacca	180
catggacaca	ctccttgaaa	gtcgaattac	tctccttgcc	tttttggttg	tgacagtta	240
tttttctggt	accttactta	cagatgtttt	tgttccctata	ctcttgatca	agagctgac	300
ccaaaacagt	gggtactgt	atcatcccta	tatgcttggc	agttatttgc	aatcgccacc	360
aggcatttgt	caaggcttct	aatcagatca	gcagactaca	actgattgac	acgtnaaatc	420
agtcaccgtt	ttttccctac	nattacaaaa	ctgccagtcc	tatatggagt	ctgatcacia	480
gactgcagtt	tcttcacaga	tctcaggaag	ttgtcgtggg	gcanaagctt	tttaaaaaca	540
tgtgattagg	gagctatctt	tatctgaata	ataac			575

<210> 12

<211> 442

<212> DNA

<213> human

<400> 12

```

gtatattkgc agtcagaggc accaaaaatg cacaccttgc aggttcctga aaaccactca      60
gtagccttaa accaaactac aaatggccat actgaatcaa ataactatat atataaaacc      120
ttgggtgtaa ataagcagac agaaaatcta aagaatcaac agactgagaa tctacttaaa      180
aggcgaagtt tcccgttatt tgacaactca aaagccaact tagatcctgg aaatagtaag      240
cattatgtat atagtacact taccaggaat cgagtttagac aaccagaaaa gccawagca      300
akatttgctg aaawgttcta aaagcatgcr caatgtgact cataacttgg aggaggatga      360
ggaggaagtt accaagaaga aactctccaa gtggcactac taccaaatca gtttccattg      420
ctgctttact tgatgtgaat aa

```

<210> 13

<211> 332

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1)...(332)

<223> n = A,T,C or G

<400> 13

```

ccaagttaca agtttttttc tagtgettat gtacgtttta agcccatgac ctacctgtgg      60
gagtgcacct acaagccgga gtgtttcatt caatctatat ctaatcttta actagagtct      120
ggagtctgga aggtttttctc tagagtcttg gaaagtttct taagtgggac ctggtacaag      180
gtatacgtgt aagaatgcct ttattattca atcagacatt aggggtctaag aaaaccacag      240
tggggtcata atgggtttgt ttctgtattc canccgttgt actcaggcac cagtttcccc      300
agttctttaa tgtttaactt ctacatacat ca

```

<210> 14

<211> 970

<212> DNA

<213> human

<220>

<221> misc_feature

<222> (1)...(970)

<223> n = A,T,C or G

<400> 14

```

aaaagctgga gctcgcgcgc ctgcaggctg acactagtgg atccaaagaa ttcggcacga      60
gaggcaccaa tgaagacatg gtgtttcgtg gaaacattga taacaacact ccatatgcta      120
actctttcac acccccata aaagctcagt atgtaagact ctatcccaa gtttgcgaa      180
gacattgcac tttgcgaatg gaacttcttg gctgtgaact gtcgggttgt tctgagcctc      240
tgggtatgaa atcaggacat atacaagact atcagatcac tgcctccagc atcttcagaa      300
cgctcaacat ggacatgttc acttggggaa caaggaaaaag ctcggtctga caagcaaggc      360
aaagtgaatg cctggacctc tggccacaat gaccagtcac aatgggtaca ggtggatctt      420
cttgttccaa ccaaatgact ggcattcata cacaaggagc taaagatttt ggtcatgtac      480
agtttgttgg ctctacaaa ctggcttaca gcaatgatgg agaactctgg actgtatacc      540
aggatgaaaa gcaaaagaaa gataagggtt tccagggaaa ttttgacaat gacactcaca      600
gaaaaaatgt catcgaccct cccatctatg cactgacac aagaatcctt ccttggctct      660
ggtacgggag gatcacattg cggtcagagc tgcctggctg cacagaggag gaatgagggg      720
aggctacatt tcacaaccct cttccctatt tccctaaaaa tatctccatg gaatgaactg      780
tgcaaaatct gtaggaaact gaatgggttt nttttttt tcatgaaaaa gtgtcctaaa      840
tatggtaggc aactaacggg ttttttaagg ggggtctaaa ctgccttttc aatgatttaa      900
ttggatttta ttttatccgc aaatctctta agtaacaaca catttaagtgt gaattacttt      960
tctctcattg

```

<210> 15

<211> 528

<212> DNA
<213> human

<220>
<221> misc_feature
<222> (1)...(528)
<223> n = A,T,C or G

<400> 15
 ttctaccctt tcttgagcca catgtttcac acaagtgtag aaaatgccag ggatccacca 60
 caagatggag atggtcagca caaacggatt ctgttcctct ttaaagtgtat tattagccac 120
 ttagcaatct ctatattctt tcaagtaacc aagctgttga ctttcttact acttgacagta 180
 gcctgtcccc aacttttcca tccagtgcctt aacctaataa actccttaac tctgccttga 240
 cctgaggaan accatgctaa ctggtgttat tttgtatgta ccctgtgcctt aattctataa 300
 cagtaaaccc catacgagcagg tgggagggag gaacaccggt gcctcggtca ctctgggggc 360
 agtttagatg ctgtgaaatt aaacctgttc taagtgtact tgtttgaatt aattgtattg 420
 taatattatt tgttgaatgt agtaattagg tatttatgaa tatattgctg taatttctga 480
 caacatccaa aaaataaaat cttcctaaat taataaaaaa aaacccaa 528

<210> 16
<211> 3831
<212> DNA
<213> human

<400> 16
 ggcacgagct gggctcctgc agagcagatc ctgtctgcgt cctccaggag gagggggtgg 60
 caggactggg gtttcccaca ggttttgggg cgggcgcgag attggcacgg tccggggctg 120
 caggcgcgca gccacgcccc tggaagtccg ccccgcccc cgcccccaac ccgctcttc 180
 ggggctttat ggcgtgaggt ttggggctgg gatccatctg gagccgagca gaaaactttt 240
 cccctcccggt tcccggtccc ttttgccttt ctgggacgcg gtggcgggcg cgctgagcg 300
 gcgactccct ctccctgcc cggttgctg cgcccggtgc cctccgaggg caggcgcgcc 360
 tggactctgc gcccggtgg cgggcgccct ctgtgagcac cggcagcggc gcatccccctg 420
 ccccgaggcc tccggtgcc cccggcgcg ggcatagggg cgccccacc ctccgtccgc 480
 ttgcacccct tgcctcccgc cccctcgcc gactcatccg ccgcgggtgg ccgcccagc 540
 cctgggatgg ggaggagagc cgcggctgcc cgcgcgggc gagattcccg ctgacgcccc 600
 cgacctgcc gccttcttcg tccgctcca gagggcgccg acgtcccgac agctcctgga 660
 gtgagaccag gactgagaac agggagaggc gacccgaccc ccaggggccc gtgctcatga 720
 cagcacacag agccgctgaa aacgactgaa gagagcaatg gatttcctgt gacatctggc 780
 tctggagagt aaaatgccaa gctatgatag caactgggtg agtgataact ggctggccg 840
 ccttgaaaaa gcaagactct gccagatcac agcagcatgt caacctcagc ccgtctcctg 900
 ctacccaaga gaagaagccc atccaggcgc ccggcccccg ggcagatgtc gtggttgttc 960
 gtggcaaaat ccggctttat tccccatctg gttttttct tatttttagga gtgctcatct 1020
 ccattatagg aattgctatg gccgttcttg gatattggcc ccaaaaagaa cattttattg 1080
 atgctgaaac aacactgtca acaaatgaaa ctcaggtcac tcggaatgaa ggcggtgtgg 1140
 tggttcgctt ctttgagcag catttgcat ctgataagat gaaaatgctt ggccattca 1200
 ccattgggat tggcattttc attttcatt gtgctaagc cattctcat gaaaaccgtg 1260
 acaaaagagc caaaatcata cacatgaggg atatctattc cacagtcatt gacattcaca 1320
 cgtaaacat caaggagcaa agggcaaatga acggcaatga cactgggttg atgggagaaa 1380
 cagaagttaa acagaatggg agctcctgtg cctcgagatt ggcagcaaat acgatcgcc 1440
 ctttctcggg ttttcggagc agttttcgaa tggacagctc cgtggaggag gatgaactta 1500
 tgttaaatga aggttaagagt tctgggcatc ttatgcccc ttgtctctct gacagctctg 1560
 tgtctgtctt tggcctctat ccacctcctt ccaagacaa tgatgataag accagcggt 1620
 ctaagaaatg tgaaaccaag tcaattgtgt catcgctcat agtgctttt acattgcctg 1680
 tgcataaact taataactgt gttattgatg agccagctat agataacatc actgaagatg 1740
 ctgacaacct caaaagttag tcaaggaaat tgtcaatgga tcccttctgt gttcctttgc 1800
 ccaacaccag tgaatccttc cagcccgta gcacagtgtt accaaggaa aattccattg 1860
 gggagtcgtt gtcgagtcag tacaagtcac ctatggctct cggacctggg gctggagagc 1920
 tcttgtctcc tggggctgcc agaagacagt ttgggtccaa tacatccttg catttgctct 1980

cgtcacactc	aaagtccttg	gacttagacc	gggggccctc	caacttaact	gttcaggcag	2040
aacaacggaa	acatccaagt	tggcctaggt	tggatcggaa	caacagcaag	ggatatatga	2100
aactagagaa	caaagaagac	ccgatggata	gggtgcttgt	gccccaggtt	gccatcaaaa	2160
aggactttac	caataaggag	aagcttctta	tgatttcaag	atctcacaat	aatttgagtt	2220
ttgaacatga	tgagtttttg	agtaacaacc	taaagagggg	aacttctgaa	acaaggtttt	2280
aatgttaaaa	gaatatatca	ttttacaagg	gtatatattt	taaaacgatt	ttcactgggtg	2340
tttccttctt	aaagtattgg	ctgtaacgtt	tttaatacaa	tggtttgtag	tgtattagaa	2400
ttggctgctt	agttctgtaa	tgaagatggt	tgtatgtttg	ggttacttgt	gactgcagta	2460
ctctatgtta	ccacacatga	ttttattttt	ctcttccctt	gaaagcatga	tctcttttat	2520
taatatgaat	gcaaaatgct	tgcattccaaa	ttaaagctta	ttttctttac	ttttaagttc	2580
tttgattgcc	ctattcataa	aatgaaatgt	ccagtatgga	aaacataagg	taccaagtg	2640
tggaccagga	gtacaaattc	agtcaccaata	ctcaatacgt	attatagatg	actatgagtg	2700
caaaccctag	gatgtgattt	tctgaataat	tgttctttgt	aggatttggt	tacattattt	2760
aaaatgaaaa	agatctagtt	ttagtgtag	ctcagtaatg	ttaattgggt	aagttcattg	2820
tgaatcttga	gttttagata	agtagttatt	tttttcaata	tcacttctgt	ttttagtgat	2880
attatatcaa	gaaacaacgt	attcaagagc	catggctgac	agtgccagat	atacttaggg	2940
ataaacatca	aaatgcaatt	atagttgcta	taacgttaga	tactcggaa	caaaatttat	3000
ttgcaagctg	acttgataaa	ctaaatgaac	caataaaaat	tgtagaaatg	gctatcctga	3060
aataattata	tacatgaaga	caatgttgac	taatgaatta	agatacatta	tatactagtt	3120
aatgctaact	agtctcagta	cctgttttta	gccatctgtt	actgtccaat	agcacctcat	3180
tcccacattc	tattttcccc	cggatattctt	tagatcctag	tatttggaat	acaatcggct	3240
aaccttgaca	tttcttttta	ccttcataatg	ccactatctc	ggtagttcaa	aaaaatttag	3300
ttcttgataa	attgccttga	agtttacctt	gtgctggaga	gccttatgat	aactccaaa	3360
actttcttac	gggtataatac	atgttggtta	ggattgtgtt	tcttagtcac	tgaagataat	3420
aaatattaaa	atggatgttt	tcacagaaaa	attttcatgt	tttcttttaa	ggtaacataa	3480
ttgtaagaat	tgtttaataa	aatactcagg	aaattctaaa	ggtttctccc	aatacctaaa	3540
catttctgaa	catcagttat	gcagttgttg	aagagcagaa	ggaggataca	tttgtttgtg	3600
ttgtcccca	aaattccacc	ttgcatttgc	atcacaaaat	tccctcaatt	gaggcagttt	3660
tctttgttag	aacattaaat	ctgtgtattg	taataagatg	ggctcaatat	tttactataa	3720
agcatttaat	aaactgttac	caatagaagt	ttgtgttctt	cacaccttgc	ctattgcttt	3780
ttaaataaaa	tgtacatttc	tgcttaaaaa	aaaaaaaaaa	aaaaaaaaaa	g	3831

<210> 17

<211> 1718

<212> DNA

<213> human

<400> 17

aatgaaagag	cttcttacc	agtgtgtgtg	cccttttgag	tatttttgtt	tttaaaataa	60
tgattgtaaa	atgtttttaca	agtaatgtaa	aagctagtat	cattctttaca	tacttctgtg	120
tttaaatattt	cattctttacc	aaaacagtta	actctttctt	tccaatcaat	ttatacaaaa	180
gaggctgctc	cagccctacc	acaggctctga	ctggcactgc	cttttgtttg	cccttgaaca	240
gggcagtgtt	gtggggactg	caaaagagaa	aacgtccagg	cgagccaggt	tgtcctcgcc	300
cacagggtcc	tgcaggctcc	atcagtcacc	gctttctatg	gcgtttgtag	ttgtgtcttt	360
taagaagtga	gtgtgattgt	ttacttgata	aatcagctca	ctctctgggtg	cttttttagag	420
aagtccctga	ttccttctta	aaacttgaat	gatagatgaa	attcacaccc	ctgcagatca	480
gaaaaacaaa	tagaagaaaa	tgagggttac	agtaacctgt	tgtctttata	taacttgcaa	540
caaaactaatt	tatttttttt	tccttttttt	gtttttgggt	ttttatgggt	ttttaaggaa	600
aatacttttc	tcctttgaag	ttttacagct	ttttgtaaat	gcgtcctgat	aatgattagg	660
aaaaatcgacc	ttttcatcca	tgatgacccat	cctcatagct	cagatttccct	ttcaaaggtag	720
tggctttctg	gatggtaatt	ccatcttaag	gtgtcagaac	tattttcaaa	tgctgccttt	780
gacagttctt	ggaattttct	gatattaagc	agttccatgc	aaatattcgt	gttttataaa	840
tagctctcat	agtctgctcc	atcttgatag	ttaaagtatt	tctgaagcgt	ttgtgtgtgt	900
gttgatcagg	ttgtgtgata	tttttgcttg	ataaagaatc	aaatttgaaa	caattaacca	960
gccagtagat	tgtctgtcag	tgaccttctg	tagtaataaa	gtttttgcca	ctgtaaaataa	1020
aaacagtatc	cgtagctatc	aggatcattg	cgcactcata	tatgctaagc	cttctgttct	1080
ctaataagaag	cctttctttt	ccattgtttc	tggataattg	tattatccaa	atgtgcttat	1140
ttctttgcct	tagcacacgt	tttatggagt	acctgttata	ctagggttga	tttgaaactg	1200

```

gtgcttgctg cagaactgtc agagcatgag gagcgctcct cctgtgggtg gacgcattca 1260
cgcaactcccc aggttgacc tgctgctggc ggtgagcagg gggttcagca gcttgaccga 1320
tgccccccga gggggctctc ccagcttaa actttgtgtg ttaaatttgt taacttttta 1380
tattaatgac tattgaaagt ggtaataaaa atttatatta taggcttcaa tgttttcatg 1440
aatgttacct aaaaagctgt gttttctttg gtcagagggtc aaaatttatg aaaaacaaaa 1500
tgctgtatga atggaaatca ttttgcaatt gagtgacact tcattgtaat tcacagtgtg 1560
aatttaattc aaactgaaat tttgtttcaa ctgaatttgt aattaactct gaatttggtt 1620
ttaatcatta gtaattttc agttgggtat ctttttaagt aaaaacaaca aataaactct 1680
gtacatgtaa aacgtgaaaa aaaaaaaaaa aaaaaaaaaa 1718

```

```

<210> 18
<211> 1873
<212> DNA
<213> human

```

```

<400> 18
aggcacgagg ccccgcgcg cggccgagtc gctgagccgc ggctgccgga cgggacggga 60
ccggctaggc tgggcgcgcc ccccgggccc cgccgtgggc atgggcgcac tggcccgggc 120
gctgctgctg cctctgctgg ccagtggtt cctgcgcgcc gccccggagc tggccccgcg 180
gcccttcacg ctgccccctc gggtgccgc ggcacgaac cgcgtagttg cggccacccc 240
gggacccggg acccctgccg agcgccacgc cgacggcttg gcgctcgccc tggagcctgc 300
cctggcgctc cccgcgggcg ccgccaactt cttggccatg gtagacaacc tgcaggggga 360
ctctggccgc ggctactacc tggagatgct gatcgggacc cccccgcaga agctacagat 420
tctcgttgac actggaagca gtaactttgc cgtggcagga accccgcaact cctacataga 480
cacgtacttt gacacagaga ggtctagcac ataccgctcc aagggtcttg acgtcacagt 540
gaagtacaca caaggaagct ggacgggctt cgttggggaa gacctcgtca ccattcccaa 600
aggcttcaat acttcttttc ttgtcaacat tgccactatt tttgaatcag agaatttctt 660
tttgccctggg attaaatgga atggaatact tggcctagct tatgccacac ttgccaaagg 720
atcaagttct ctggagacct tcttcgactc cctggtgaca caagcaaaaa tccccaacgt 780
tttctccatg cagatgtgtg gagccggctt gcccggtgct ggatctggga ccaacggagg 840
tagtcttgct ttgggtggaa ttgaaccaag tttgtataaa ggagacatct ggtatacccc 900
tattaaggaa gagtgtact accagataga aattctgaaa ttggaaattg gaggccaaag 960
ccttaatctg gactgcagag agtataacgc agacaaggcc atcgtggaca gtggcaccac 1020
gctgctgcgc ctgccccaga aggtgtttga tgcggtggtg gaagctgtgg cccgcgcac 1080
tctgattcca gaattctctg atgggtttctg gactgggtcc cagctggcgt gctggacgaa 1140
ttcggaaaaa ccttggctctt acttccttaa aatctccatc tacctgagag atgagaactc 1200
cagcaggtca ttccgtatca caatcctgcc tcagctttac attcagccca tgatgggggc 1260
cggcctgaat tatgaatgtt accgattcgg catctcccca tccacaaatg cgctgggtgat 1320
cgggtgccacg gtgatggagg gcttctacgt catcttcgac agagcccaga agagggtggg 1380
cttcgcagcg agcccctgtg cagaaattgc aggtgctgca gtgtctgaaa tttccgggcc 1440
tttctcaaca gaggatgtag ccagcaactg tgtccccgct cagtctttga gcgagcccat 1500
tttgtggatt gtgtcctatg cgctcatgag cgtctgtgga gccatcctcc ttgtcttaat 1560
cgtcctgctg ctgctgccgt tccggtgtca gcgtcgcccc cgtgaccctg aggtcgtcaa 1620
tgatgagtc tctctggtca gacatcgctg gaaatgaata gccaggcctg acctcaagca 1680
accatgaact cagctattaa gaaaatcaca tttccagggc agcagccggg atcgatgggtg 1740
gcgctttctc ctgtgcccac ccgtcttcaa tctctgttct gctcccagat gccttctaga 1800
ttcactgtct tttgattctt gattttcaag ctttcaaact ctccctactt ccaagaaaaa 1860
aaaaaaaaa aaa 1873

```

```

<210> 19
<211> 518
<212> PRT
<213> human

```

```

<400> 19
Met Gly Ala Leu Ala Arg Ala Leu Leu Leu Pro Leu Leu Ala Gln Trp
1           5           10           15
Leu Leu Arg Ala Ala Pro Glu Leu Ala Pro Ala Pro Phe Thr Leu Pro

```

SUBSTITUTE SHEET (rule 26)

485 490 495
 Gln Arg Arg Pro Arg Asp Pro Glu Val Val Asn Asp Glu Ser Ser Leu
 500 505 510
 Val Arg His Arg Trp Lys
 515

<210> 20
 <211> 31
 <212> DNA
 <213> human

<400> 20
 acgactcact atagggcttt ttttttttta a 31

<210> 21
 <211> 26
 <212> DNA
 <213> human

<400> 21
 acaatttcac acaggacgac tccaag 26



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/57, C07K 14/47, C12N 9/64, C07K 16/18, C12Q 1/68, G01N 33/50	A3	(11) International Publication Number: WO 99/34004 (43) International Publication Date: 8 July 1999 (08.07.99)
(21) International Application Number: PCT/US98/27608 (22) International Filing Date: 24 December 1998 (24.12.98) (30) Priority Data: 60/070.112 31 December 1997 (31.12.97) US (71) Applicant: CHIRON CORPORATION [US/US]; 4560 Horton Street - R440, Emeryville, CA 94608 (US). (72) Inventors: XIN, Hong; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US). GIESE, Klaus; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US). (74) Agents: POTTER, Jane, E., R. et al.; Chiron Corporation, Intellectual Property - R440, P.O. Box 8097, Emeryville, CA 94662-8097 (US).	(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> (88) Date of publication of the international search report: 4 November 1999 (04.11.99)	
(54) Title: METASTATIC BREAST AND COLON CANCER REGULATED GENES (57) Abstract Gene sequences as shown in SEQ ID NO:1-18 have been discovered and isolated, and found to be significantly associated with metastatic spread of breast and colon cancer cells to other organs. Methods are provided for determining the risk of metastasis of a breast or colon tumor, which involve determining whether a tissue sample from a tumor expresses a polypeptide encoded by a gene as shown in SEQ ID NOS:1-18, or a substantial portion thereof. One of the gene sequences encodes a novel aspartyl protease termed CSP56, which can be used to provide reagents and methods for determining which tumors are likely to metastasize and for suppressing metastases of these tumors. Clinicians can use this information to predict which tumors will metastasize to other organs and to provide relevant therapies to appropriate patients.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroun	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/27608

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/57 C07K14/47 C12N9/64 C07K16/18 C12Q1/68
G01N33/50

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K C12Q G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	CHEN, WEN-TIEN ET AL: "Membrane proteases as potential diagnostic and therapeutic targets for breast malignancy" BREAST CANCER RES. TREAT. (1994), 31(2/3), 217-26 CODEN: BCTRD6;ISSN: 0167-6806, XP002101849 the whole document ---	1,3,8, 10,11, 13,16, 17,19, 21,23, 25,27, 29-32, 34,36, 39,40, 42,43

☒ Further documents are listed in the continuation of box C

☒ Patent family members are listed in annex.

* Special categories of cited documents

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z document member of the same patent family

Date of the actual completion of the international search

4 May 1999

Date of mailing of the international search report

17.09.99

Name and mailing address of the ISA

European Patent Office, P.B. 5813 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Mennessier, T

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/27608

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	<p>DUFFY M J: "Proteases as prognostic markers in cancer." CLINICAL CANCER RESEARCH. (1996 APR) 2 (4) 613-8. REF: 73 JOURNAL CODE: C2H. ISSN: 1078-0432., XP002101850 United States</p> <p>the whole document</p>	<p>1.3,8, 10,11, 13,16, 17,19, 21,23, 25,27, 29-32, 34,36, 39,40, 42,43</p>
P,X	<p>EP 0 848 062 A (SMITHKLINE BEECHAM PLC ;SMITHKLINE BEECHAM CORP (US)) 17 June 1998 (1998-06-17)</p> <p>page 6 - page 7 page 15 - page 18</p>	<p>1.3,8, 10,11, 13,16, 17,19, 21,23, 25,27, 29-32, 34,36, 39,40, 42,43</p>

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,40,42,43 (all partly)

This first group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:1.

2. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,41,42,43 (all partly)

This second group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:2.

3. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,41-43 (all partly)

This third group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:3.

4. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,41-43 (all partly)

This fourth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:4.

5. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,36,
39 (all partly)

This fifth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:5.

6. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,41,42,43 (all partly)

This sixth group of inventions consists of the inventions as

INTERNATIONAL SEARCH REPORT

International application No

PCT/US 98/27608

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

- 1 ☐ Claims Nos.
because they relate to subject matter not required to be searched by this Authority, namely
- 2 ☐ Claims Nos.
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful international Search can be carried out, specifically
- 3 ☐ Claims Nos.
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows.

See additional sheet

- 1 ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
- 2 ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
- 3 ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.
- 4 ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.

1, 3, 5, 8, 10, 11, 13, 16, 17, 19, 21, 23, 25, 27, 29-32, 34, 36, 39, 40, 42, 43 (all partly)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest
- ☐ No protest accompanied the payment of additional search fees

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:6.

7. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36, 39,41,42,43 (all partly)

This seventh group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:7.

8. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36, 39,41,42,43 (all partly)

This eighth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:8.

9. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36, 39,40,41,42,43 (all partly)

This ninth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:9.

10. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36, 39,41,42,43 (all partly)

This tenth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:10.

11. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36, 39,40,42,43 (all partly)

This eleventh group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:11.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

12. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,41,42,43 (all partly)

This twelfth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:12.

13. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,41,42,43 (all partly)

This thirtieth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:13.

14. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,36,
39 (all partly)

This fourteenth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:14.

15. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,36,
39,41,42,43 (all partly)

This fiftieth group of inventions consists of the inventions as defined in the above claims, insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:15.

16. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34-36,
39,40,42,43 (all partly)

This sixtieth group of inventions consists of the inventions as defined in the above claims insofar as the claimed subject-matter concerns the sequence referred to as SEQ ID NO:16.

17. Claims: 1,3,5,8,10,11,13,16,17,19,21,23,25,27,29-32,34,35,
36,39,40,42,43 (all partly)

This seventieth group of inventions consists of the